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(54) Title: METHOD FOR UTILIZING NEUTRAL LIPIDS TO MODIFY IN VIVO RELEASE FROM MULTIVESICULAR LIPO- SOMES (57) Abstract The rate of release of encapsulated active compound from a multivesicular liposomal (MVL) formulation is modified by selection of a neutral lipid component. A family of MVL formulations containing different slow:fast release neutral lipid molar ratios displays different release rates depending upon the molar ratio of the fast release neutral lipid to the slow release neutral lipid in each member. Incubation in plasma or a plasma-like medium at <i>in vivo</i> temperatures so as to obtain a release rate curve for each allows selection from among the members of the family of a liposomal formulation with a desired rate of release <i>in vivo</i> .		

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**METHOD FOR UTILIZING NEUTRAL LIPIDS TO MODIFY
IN VIVO RELEASE FROM MULTIVESICULAR LIPOSOMES**

5 Background of the Invention

This invention relates to liposomal formulations of compounds such as drugs. More particularly this invention relates to methods of modifying the *in vivo* rate of release of encapsulated compounds from
10 multivesicular liposomes by the choice of the neutral lipid in the liposomal formulation.

When phospholipids and many other amphipathic lipids are dispersed gently in an aqueous medium they swell, hydrate, and spontaneously form multilamellar
15 concentric bilayer vesicles with layers of aqueous media separating the lipid bilayers. These systems are commonly referred to as multilamellar liposomes or multilamellar vesicles (MLV), and usually have diameters of from 0.2 to 5 μm . Sonication of MLV results in the
20 formation of small unilamellar vesicles (SUV) bounded by a single lipid bilayer with diameters usually in the range of from 20 to 100 nm, containing an aqueous solution. Multivesicular liposomes (MVL) differ from MLV and SUV in the way they are manufactured, in the random,
25 non-concentric arrangement of aqueous-containing chambers within the liposome, and in the inclusion of neutral lipids necessary to form the MVL.

Various types of lipids differing in chain length, saturation, and head group have been used in liposomal
30 drug formulations for years, including the unilamellar, multilamellar, and multivesicular liposomes mentioned

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above. The neutral lipids used in the manufacture of multivesicular liposomes to date have been primarily limited to triolein and tricaprylin.

One of the major goals of the field is to develop liposomal formulations for controlled *in vivo* release of drugs and other active agents of interest. Certain drugs need to be released fairly rapidly upon emplacement of the liposomal depot, and others require a relatively slow rate of release over a sustained period of time.

Heretofore, the rate of release of a biologically active compound from a liposomal formulation has been modified by selection of the amphipathic lipid, the accepted membrane forming lipid, or by manipulation of the phospholipid/cholesterol molar ratio. Alternatively, such compounds as an acid or an osmolality spacer have been included in the aqueous solution for encapsulation to aid in modifying the rate of release of the encapsulated biologically active compound.

The control of release rates from liposomal formulations is complicated by the fact that many biologically active agents, such as proteins, need to be stored at reduced temperatures, i.e., about 4°C, to retain full activity. Unfortunately, some liposomal formulations that display excellent release rates at *in vivo* temperatures disintegrate rather rapidly at such storage temperatures.

Thus, the need exists for more and better methods for selecting liposomal formulations that maximize control over the rate of release of the encapsulated active compound while simultaneously affording shelf life stability for long periods of time at storage temperatures of about 4°C, for example 2 to 10°C.

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Summary of the Invention

In general, the invention features a method for modifying the rate of release of a biologically active compound, such as a drug, that is encapsulated in a multivesicular liposomal formulation by utilizing in the formulation a neutral lipid component having a selected molar ratio of a slow release neutral lipid to a fast release neutral lipid, wherein the proportion of the fast release neutral lipid in the molar ratio is increased to increase the rate of release of the biologically active compound. Alternatively, the proportion of the slow release neutral lipid in the molar ratio can be increased to decrease the rate of release of the biologically active compound. Generally, the slow:fast neutral lipid molar ratio is in the range from about 1:1 to 0:1, for example 1:4 to 1:100, or 1:4 to 1:27, and the molar ratio of the neutral lipid component to the total lipid component (all the lipids in the liposome) is in the range from about 0.01 to about 0.21.

For modifying the *in vivo* release rate, the melting point of the neutral lipid component preferably is at or below the *in vivo* temperature at which the formulation is to be used, as well as at or below the temperature at which the formulation is to be stored.

Slow release neutral lipids useful in the new method of this invention are, for example, triolein, tripalmitolein, trimyristolein, trilaurin, and tricaprinn with triolein being most preferred. Useful fast release neutral lipids include, e.g., tricaprillin and tricaproin, and mixtures thereof. However, tricaproin and other similar lipids are usually not used as the sole neutral lipid in a formulation of multivesicular liposomes intended for use *in vivo*.

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In another embodiment, the present invention provides a liposomal composition comprising a therapeutically effective amount of a biologically active compound encapsulated in a multivesicular liposome formulation wherein the formulation comprises a neutral lipid component comprising a molar ratio of slow release neutral lipid to fast release neutral lipid from about 1:1 to 1:100, for example from about 1:3 to 1:54, 1:4 to 1:27, or from about 1:4 to 1:18.

10 In yet another embodiment, the invention features a method for selecting a multivesicular liposomal formulation for encapsulating a selected biologically active compound so as to obtain a desired release rate profile of the active compound *in vitro* and/or a desired therapeutic release rate *in vivo*. In this embodiment of the invention, a family of MVL formulations that encapsulate the selected biologically active compound is prepared wherein each member of the family utilizes a neutral lipid component having a different slow:fast neutral lipid molar ratio, generally in the range from 1:0 to 0:1. For example, a family of formulations utilizing slow:fast neutral lipid molar ratios of 1:0, 1:1, 1:4, 1:18, 1:27, 1:100, 0:1 can be prepared. Each member of the family of formulations is incubated in the medium in which the desired rate of release is to be obtained, i.e., either in a storage medium at storage temperature or in human plasma, a plasma-like medium, or in a physiological medium into which the physiologically active substance is to be released, such as cerebrospinal fluid (CSF) at body temperature. By this means a release rate profile is obtained for each formulation. Then the formulation having the slow:fast neutral lipid ratio that yields the desired release rate profile under the desired

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conditions for the selected biologically active substance is selected.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The practice of this invention provides the advantage that a formulation of multivesicular liposomes can be selected to have relatively rapid release *in vivo* that does not compromise the desire of slow release at storage conditions. The method of the invention, therefore, provides a rationale for obtaining a desired release rate through selection of the neutral lipid component used in manufacture of the MVL without compromising the desire for the formulation to have a slow release at storage conditions. Further, the control over the rate of release under *in vivo* conditions operates more or less independently of the composition of the aqueous phase encapsulated or the combination of the other lipids in the formulations.

This finding is particular to multivesicular liposomes since other types of liposomes do not contain neutral lipids in the lipid component and/or do not

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incorporate the neutral lipids into closely packed, non-concentric vesicles.

Other features and advantages of the invention will be apparent from the following detailed description,
5 and from the claims.

Brief Description of the Drawings

Figure 1 is a graph comparing the release rates of recombinant, human granulocyte colony stimulating factor (rhu G-CSF) from multivesicular liposomes (MVL)
10 formulated with different molar ratios of triolein to tricaprylin (∇ , 100:0; Δ , 50:50; \circ , 25:75; \square , 10:90; \diamond , 0:100) as the neutral lipid when incubated at 37°C in 60% human plasma in saline solution.

Figure 2 is a graph showing the results of a
15 pharmacodynamic study comparing the duration of the rhu G-CSF dependent elevation in peripheral blood leucocyte number of golden Syrian hamsters following subcutaneous injection of 100 ug/kg of rhuG-CSF in saline solution (∇), in a triolein-only, 100:0, MVL formulation (\circ), in a
20 triolein-only MVL formulation solubilized pre-injection with Tween™ 20 (Δ), and in a tricaprylin-only, 0:100, MVL formulation (\square).

Figure 3 is a graph comparing the release rates of rhu GM-CSF from MVL formulated with different molar
25 ratios of triolein to tricaprylin (Δ , 100:0; \circ , 25:75; \square , 10:90) as the neutral lipid when incubated at 37°C in 60% human plasma in saline solution.

Figure 4 is a graph showing a pharmacokinetic study of levels of rhu GM-CSF in venous blood, plasma
30 samples of mice following a subcutaneous injection of 1 mg/kg rhu GM-CSF in the triolein-only, 100:0, MVL formulation (\circ) and in the 25:75, triolein:tricaprylin

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MVL formulation (\square). The rhu GM-CSF level in EDTA-plasma was determined by ELISA. LOQ is the limit of quantitation.

Figure 5 is a graph showing the release rates of
5 rhu IGF-1 from multivesicular liposomes formulated with various triglycerides as the neutral lipid component when incubated at 37°C in 60% human plasma in saline solution. The neutral lipids used in MVL manufacture were: (\square) triolein, (O) tripalmitolein, (Δ) tricaprylin, (∇)
10 trilaurin, and (\diamond) tricaprln.

Figure 6 is a graph showing the results of a pharmacokinetic study of levels of rhu IGF-1 in serum of rats following subcutaneous injection of 6.25 mg/kg rhu IGF-1 in the tripalmitolein MVL formulation (O) and in
15 the tricaprylin MVL formulation (Δ). The rhu IGF-1 level in serum was determined by ELISA.

Figure 7 is a graph showing the release of rhu insulin and coencapsulant ^{14}C -sucrose from MVL manufactured with the neutral lipid, triolein or
20 tricaprylin when incubated at 37°C in saline solutions containing 60% human plasma. (\square) sucrose retained by triolein MVL formulation; (O) insulin retained by triolein MVL formulation; (Δ) sucrose retained by tricaprylin MVL formulation; and (∇) insulin retained by
25 tricaprylin MVL formulation.

Figure 8 is a graph comparing the release rates of morphine from MVL formulated with different molar ratios of triolein to tricaprylin as the neutral lipid when incubated at 37°C in 60% human plasma in saline solution.
30 (\square) 10:0, triolein:tricaprylin, 10 batches; (\odot , O) 1:4, triolein:tricaprylin, 2 batches; (Δ) 1:9, triolein:tricaprylin; (∇) 0:10, triolein:tricaprylin.

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Figure 9 shows results of a pharmacokinetic study of morphine MVL formulations manufactured with different molar ratios of triolein to tricaprylin and injected into the epidural space of Beagle dogs. Morphine release by the MVL was determined by measuring the level of morphine in the adjacent, dura membrane-separated, cerebral spinal fluid. (\diamond) 5 mg morphine (sulfate) in saline solution; (∇) 40 mg of morphine in the 10:0, triolein:tricaprylin MVL formulation; (O) 20 mg of morphine in the 1:4, triolein:tricaprylin MVL formulation; (\square) 20 mg of morphine in the 1:9, triolein:tricaprylin MVL formulation; and (Δ) 20 mg of morphine in the 0:10, triolein:tricaprylin MVL formulation.

Figure 10 is a graph summarizing in an Arrhenius transformation the results of a study determining the effect of storage temperature on release rate of morphine from 2 batches of the 1:9, triolein:tricaprylin MVL formulation. The MVL were stored in normal saline solution at 4°C, 26°C, 37°C, and 41°C. The ordinate is the rate of morphine release from MVL expressed in terms of amount released per day as a percentage of total amount of morphine in the MVL suspension; the abscissa is $1/\text{storage temperature} \times 10^4$ ($^{\circ}\text{K}$)⁻¹.

Figure 11 is a graph showing cytosine arabinoside (AraC) retained in MVL incubated in human plasma at 37°C. The MVL formulations used as the neutral lipid component (\square) triolein only; (O) 1:4, triolein:tricaprylin; (∇) 1:9, triolein:tricaprylin; (x) 1:18 triolein:tricaprylin; (Δ) 1:27, triolein:tricaprylin; and (\diamond) tricaprylin only.

Figure 12 is a graph showing the effect the neutral lipid ratio on release of cytosine arabinoside from MVL incubated in human plasma at 37°C when the MVL

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were formulated with the major phospholipid component, DOPC, replaced with DSPC. The formulations were (□) DOPC and triolein only; (Δ) DOPC 1:9, triolein:tricaprylin; (◇) DOPC and tricaprylin only; (O) DSPC and triolein only; (∇) DSPC 1:9, triolein:tricaprylin; and (+) DSPC and tricaprylin only.

Figure 13 is a graph showing the effect of the neutral lipid ratio on release of ¹⁴C-sucrose from MVL incubated in human plasma at 37°C when the MVL were formulated with the major phospholipid component, DOPC, replaced with DSPC. The DOPC formulations were (□) triolein only; (Δ) 1:9, triolein:tricaprylin; and (O) tricaprylin only. The DSPC formulations were (∇) triolein only; (+) 1:9, triolein:tricaprylin; and (◇) tricaprylin only.

Figure 14 is a graph showing the effect of using tricaproin as the neutral lipid component alone or in combination with triolein on the release of ¹⁴C-sucrose from MVL incubated in human plasma at 37°C. The formulations were (O) triolein only; (Δ) 1:4 triolein:tricaproin; (∇) 1:9 triolein:tricaproin; (◇) 1:18 triolein:tricaproin; and (+) tricaproin only.

Figure 15 is a graph showing the in vitro release of a 15-mer oligonucleotide from MVL prepared with triolein (□) or tricaprylin (O) as the neutral lipid. The MVL were incubated in rat cerebral spinal fluid (CSF) at 37°C.

Figure 16 is a graph showing the release during incubation in human plasma of ¹⁴C-sucrose from MVL also containing E. coli plasmid PBR 322 and lysine-hydrochloride. Sucrose release was used as a surrogate

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indicator of plasmid release. The MVL were manufactured with either (O) triolein or (Δ) tricaprylin as the neutral lipid.

Figure 17 is a graph showing the release of 14C-
5 sucrose during incubation in human plasma of MVL
manufactured with tripalmitolein and tricaprylin as the
neutral lipid component. The neutral lipid ratio used in
the MVL formulations were (O) tripalmitolein only; (+)
1:1 tripalmitolein:tricaprylin; (◇) 1:2
10 tripalmitolein:tri-caprylin; (▽) 1:4
tripalmitolein:tricaprylin; (Δ) 1:9
tripalmitolein:tricaprylin; and (□) tricaprylin only.

Figure 18 is a graph showing the amount of
tetracaine remaining at the subcutaneous injection site
15 of mice injected with tetracaine containing MVL
manufactured with a neutral lipid component of either (*)
triolein; (X) 10:90, triolein:tricaprylin; (+) 5:95,
triolein:tricaprylin; (◇) 2:98, triolein:tricaprylin;
(▽) 1:99, triolein:tricaprylin; or (O) tricaprylin.

20 Figures 19A-D are graphs showing the release of
MVL-encapsulated, biologically active compounds during
storage in normal saline at temperatures of 2 - 8
(nominal 4), 25, 32, and 37°C. The MVL were manufactured
with either tricaprylin (Figures 19A and 19C); or
25 tricaprln (Figures 19B and 19D); as the neutral lipid
component and with either cytosine arabinoside (Figures
19A and 19B); or morphine, Figures 19C and 19D) as the
biologically active compound.

Figure 20 is an Arrhenius plot of the graphs in
30 Figures 19A and 19B showing the dependence of cytosine
arabinoside release rate on the storage temperature of
MVL manufactured with (□) tricaprylin, melting point (mp)
8°C; or (O) tricaprln, mp 31.5°C, as the neutral lipid

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component. The abscissa is the inverse of storage temperature, °K.

Detailed Description

A method is provided for modifying the rate of release of a biologically active compound, such as a drug, encapsulated in a multivesicular liposomal formulation by selection of the neutral lipid, or combination of neutral lipids, used to manufacture the multivesicular liposomes (MVL).

There are at least three types of liposomes. The term "multivesicular liposomes (MVL)" as used throughout the specification and claims means man-made, 1-200 μm particles partially comprised of lipid membranes enclosing multiple non-concentric aqueous chambers. In contrast, "multilamellar liposomes or vesicles" (MLV) have multiple "onion-skin" concentric membranes, in between which are shell-like concentric aqueous compartments. Multilamellar liposomes characteristically have mean diameters in the micrometer range, usually from 0.5 to 25 μm . The term "unilamellar liposomes or vesicles (ULV)" as used herein refers to liposomal structures having a single aqueous chamber, usually with a mean diameter range from about 20 to 500 nm.

Multilamellar and unilamellar liposomes can be made by several relatively simple methods. The prior art describes a number of techniques for producing ULV and MLV (for example U.S. Patent No. 4,522,803 to Lenk; 4,310,506 to Baldeschweiler; 4,235,871 to Papahadjopoulos; 4,224,179 to Schneider, 4,078,052 to Papahadjopoulos; 4,394,372 to Taylor 4,308,166 to Marchetti; 4,485,054 to Mezei; and 4,508,703 to Redziniak).

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By contrast, production of multivesicular liposomes requires several process steps. Briefly, the preferred method for making MVL is as follows: The first step is making a "water-in-oil" emulsion by capturing in
5 a lipid component composed of at least one amphipathic lipid and at least one neutral lipid in one or more volatile organic solvents for the lipid component, an immiscible first aqueous component and a biologically active substance to be encapsulated, and optionally
10 adding, to either or both the lipid component and the first aqueous component, an acid or other excipient for modulating the release rate of the encapsulated biologically active substances from the MVL. The mixture is emulsified, and then mixed with a second immiscible
15 aqueous component to form a second emulsion. The turbulence required for formation of the second emulsion is provided either mechanically, by ultrasonic energy, nozzle atomization, and the like, or by combinations thereof, to form solvent spherules suspended in the
20 second aqueous component. The solvent spherules contain multiple aqueous droplets with the substance to be encapsulated dissolved in them (see Kim *et al.*, *Biochem. Biophys. Acta*, 728:339-348, 1983). For a comprehensive review of various methods of ULV and MLV preparation,
25 refer to Szoka, *et al.* *Ann. Rev. Biophys. Bioeng.* 9:465-508, 1980.

The term "solvent spherule" as used throughout the specification and claims means a microscopic spheroid droplet of organic solvent, within which are multiple
30 smaller droplets of aqueous solution. The solvent spherules are suspended and totally immersed in a second aqueous solution.

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The term "neutral lipid" means an oil or fat that has no membrane-forming capability by itself and lacks a hydrophilic "head" group.

The term "amphipathic lipid" means a molecule that
5 has a hydrophilic "head" group and hydrophobic "tail" group and has membrane-forming capability.

The term "zwitterionic lipid" means an amphipathic lipid with a net charge of zero at pH 7.4.

The term "anionic lipid" means an amphipathic
10 lipid with a net negative charge at pH 7.4.

The term "cationic lipid" means an amphipathic lipid with a net positive charge at pH 7.4.

As used herein, the "shelf life" of a liposomal formulation is related to the rate of release of the
15 encapsulated substance from a liposomal formulation in a storage solution, for instance normal saline (0.9% sodium chloride), at a storage temperature, for instance at 4°C.

In general, for making multivesicular liposomes, it is required that at least one amphipathic lipid and
20 one neutral lipid be included in the lipid component. The amphipathic lipids can be zwitterionic, anionic, or cationic lipids. Examples of zwitterionic amphipathic lipids are phosphatidylcholines, phosphatidylethanolamines, sphingomyelins etc. Examples
25 of anionic amphipathic lipids are phosphatidylglycerols, phosphatidylserines, phosphatidylinositols, phosphatidic acids, etc. Examples of cationic amphipathic lipids are diacyl trimethylammoniumpropane and ethyl phosphatidylcholine. Examples of neutral lipids are
30 triolein and tripalmitolein, trimyristolein and tricaprylin. In the new method, the release rate of the biologically active compound is modified by utilizing in manufacture of the multivesicular liposomes a neutral

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lipid component that provides the desired rate of release in the type of fluid in which the MVL are to be used.

For *in vivo* use, therefore, the release rate of the MVL must be determined in plasma or a plasma-like medium

5 because the release rate of the biologically active compound from the MVL for certain neutral lipids can differ greatly depending on whether the release is into saline or plasma.

As used herein, the term "neutral lipid component"
10 means the neutral lipid, or mixture of neutral lipids, used in manufacture of the multivesicular liposomes.

As used herein, the term "plasma-like medium," means a synthetic solution that includes in addition to normal saline, at least some of the protein or lipid
15 constituents of blood plasma or components of other biological fluids, such as cerebro-spinal fluid (CSF), or interstitial fluids. For instance, normal saline containing citrated human plasma or bovine serum albumin (BSA) is an example of a "plasma-like medium" as the term
20 is used herein.

The term "*in vivo* conditions" means actual injection or emplacement of MVL into a living body, and includes so-called "*ex vivo*" incubation of MVL in plasma or a plasma-like medium at body temperature (i.e., 37°C
25 for humans).

Although the neutral lipid component can comprise a single neutral lipid, generally the neutral lipid component comprises a mixture of a slow release neutral lipid and a fast release neutral lipid in a molar ratio
30 range from about 1:1 to 1:100, e.g., from about 1:4 to 1:18, wherein the rate of release of the biologically active compound decreases in proportion with the increase in the ratio of the slow release neutral lipid to the

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fast release neutral lipid. For convenience, the molar ratio of the slow release neutral lipid to the fast release neutral lipid is referred to herein as "the slow:fast neutral lipid molar ratio."

5 The "slow release neutral lipid" used in the practice of this invention can be selected from triglycerides having monounsaturated fatty acid ester moieties containing from about 14 to 18 carbons in the acyl chain and generally having a molecular weight from
10 about 725 to 885, and those with saturated fatty acid ester moieties containing from about 10 to 12 carbons in the acyl chain and generally having a molecular weight from about 725 to 885; and mixtures thereof. Cholesterol esters such as cholesterol oleate and esters of propylene
15 glycol. The preferred slow release neutral lipids for use in the method of this invention are triolein, tripalmitolein, trimyrystolein, trilaurin, and tricaprinn, with triolein or tripalmitolein being most preferred. When *in vivo* use is contemplated, trilaurin (mp 46.5°C)
20 and other neutral lipids with a melting point above 37°C are generally used in the practice of this invention only in mixture with one or more other neutral lipids wherein the mixture has a melting point temperature at or below, and preferably below 37°C. One skilled in the art will
25 know how to determine the melting point of a mixture of lipids, such as a mixture of triglycerides.

 The "fast release neutral lipid" used in the practice of this invention can be selected from triglycerides having monounsaturated fatty acid ester
30 moieties containing from about 6 to 8 carbons in the acyl chain and having a molecular weight from about 387 to 471, and mixtures thereof. However, it has surprisingly been discovered that the use of a neutral lipid component

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in MVL containing one or more neutral lipids with an acyl chain of six or less carbons (especially use of tricaproin as the sole neutral lipid) results in rapid release of the encapsulated compounds upon contact with the *in vivo* environment. Therefore neutral lipids with an acyl chain of six or less carbons should be used only in combination with one or more neutral lipids having a longer chain acyl moiety. The preferred fast release neutral lipids are tricaprylin, and mixtures of tricaprylin and tricaproin, or mixed chain C₆ to C₈ triglycerides. Propylene glycol diesters with eight or ten carbon acyl moieties, cholesterol oleate, and cholesterol octanoate can also be used as neutral lipids.

A factor of equal importance to the molar ratio of neutral lipids is selection of a neutral lipid component having a melting point below the temperature at which the MVL is to be stored and/or used. As many biologically active compounds highly desirable in therapeutic applications require low storage temperature to prevent rapid deterioration, the neutral lipid component should be selected to have a melting point below the desired storage temperature as well as below the temperature at which the formulation will be used *in vivo*.

The results of storage temperature tests were determined by inspecting the particles microscopically and measuring by chemical assay the amounts of encapsulated material released. As is seen in these studies (Figure 20), MVLs stored at a temperature below the melting point of the neutral lipid component undergo a structural reorganization of the membranes that is, in some cases, accompanied by rapid release ("dumping") of the encapsulated contents. This phenomenon is referred to herein as "the melting point effect." The time to

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onset of the melting point effect depends upon the neutral lipid composition and the ingredients of the encapsulated aqueous phase. Certain encapsulated compounds, such as IL-2, appear to interact with MVLs in such a way as to delay by hours or even days, the onset of "freezing" at temperatures below the melting point of the neutral lipid, perhaps by influencing formation in the MVL of an intermediate, "meta-stable" state. However, even formulations with such delayed onset eventually undergo the morphological transition characteristic of the melting point effect. Examples 15 and 16 below illustrate the melting point effect on the release rates of encapsulated biologically active compounds from MVL stored at a temperature below the melting point (Figures 19A-D and 20). To distinguish between the two states of morphological transition, the delayed onset effect is referred to herein as "the temperature effect."

In selecting the neutral lipid component in accordance with the practice of this invention, it is generally preferred that the neutral lipid component have a melting point at or above the temperature at which the MVL are to be stored and/or used (to prevent rapid loss of the encapsulated active compound either during storage or during *in vivo* use) due to the melting point effect. The melting point temperature of a neutral lipid component comprising a mixture of neutral lipids, and hence the temperature of the melting point effect on MVL containing the neutral lipid component, can readily be determined by preparing the mixture of interest and subjecting it to progressively lower temperatures until the mixture is observed to "freeze." One method of performing this procedure is disclosed in J.B. Rossell,

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Advances in Lipid Research 5: 353-408, 1967. One skilled in the art will be aware of other approaches that can be used to obtain this information. However, it should be remembered that the melting point effect upon the liposomal formulation as a whole can be influenced by the other lipids in the MVL as well as by the ingredients in the first aqueous solution.

Table 1 below lists the number of carbons in the acyl chains, the molecular weight, and melting point temperatures of representative neutral lipids that can be used in the practice of this invention.

Table 1

The Physical Properties of Neutral Lipids					
	Neutral lipid	Fatty Acid Ester	Mol. Wt.	Viscosity cP	M.P. °C
15	<u>TriGlycerides</u>				
	Triolein	C18:1 9C	885	74	5
	Tripalmitolein	C16:1 9C	801		≤5
20	Trimyristolein	C14:1 9C	725	≤5	
	Trilaurin	C12	639		45.5
	Tricaprin	C10	555		31.5
	Tricaprylin	C8	471	20-28	8.3
25	Tricaproin	C6	387		≤0
	Captex 355	C8,C10 mixed	Avg. 496		≤5
	<u>Propylene Glycol Diester</u>				
	Captex 200	C8,C10 mixed	Avg. 345	9-13	≤5
30	<u>Cholesterol Ester</u>				
	Cholesterol Oleate	C18:1 9C	651		44-47
	Cholesterol Octanoate	C8	512		110

In one method of the invention, the release rate of the biologically active compound is modified by utilizing in manufacture of the multivesicular liposomes a neutral lipid component comprising triolein or tripalmitolein, or a mixture thereof, as the slow release neutral lipid, and selecting a molar ratio of the slow release neutral lipid to a fast release neutral lipid in the range from about 1:0 to 0:1. The rate of release of

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the active compound increases with the increase in the proportion of the fast release neutral lipid in the molar ratio. Generally the molar ratio of the neutral lipid component to the sum of all the lipids in the MVL

5 formulation is in the range from about 0.01 to about 0.21. The preferred fast release neutral lipid for use in such formulations with triolein and/or tripalmitolein is tricaprylin.

In addition to the melting point effect, which is
10 contributed in part by the neutral lipid component, the characteristics of the aqueous phase encapsulated in the MVL, particularly the chemical interaction of the active compound with the lipids in the MVL, can also influence the rate of release of the biologically active compound.
15 To take this additional factor into account during formulation, in one embodiment this invention provides a method of tailoring the neutral lipid component to the aqueous phase of interest.

In the first step of the method, to determine how
20 the neutral lipid component functions with any specific aqueous phase (i.e., one containing a biologically active compound of interest), a family of MVL formulations is made containing the aqueous phase of interest, wherein each member of the family of formulations contains a
25 different slow:fast release neutral lipid molar ratio of the selected slow and fast release neutral lipids such that the family as a whole represents a graded progression of such ratios, for example 1:1, 1:2, 1:4, 1:9, 1:18; 1:27, 1:100, etc.

30 The *in vivo* release rates corresponding to the various slow:fast neutral lipid molar ratios embodied in the individual members of the family of MVL formulations is determined by separately incubating each member of the

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family *in vitro* in plasma or a plasma-like medium at *in vivo* temperature, i.e. 37°C for humans, for a period of hours, or even days.

Any of the methods illustrated in the Examples, or
5 others known to one of skill in the arts, can be used to determine at progressive time points the cumulative amount of one or more substance(s) encapsulated with the aqueous phase that has been released during incubation. For ease in making this determination, a radioactive
10 substance, such as ¹⁴C sucrose, can be included in the aqueous phase at the time of encapsulation. However, it is preferred to select the biologically active compound of interest as the substance whose release rate is monitored and recorded. It is recommended that the
15 release rate information be obtained in this manner for each member of the family of formulations being tested.

From the release rate information determined by this procedure, a graph showing a release curve, or "release rate profile" can be plotted for each member of
20 the family of formulations to show its individual *in vivo* release characteristics, with the ordinate of the graph indicating the cumulative amount of the substance of interest that has either been released or retained, and the abscissa indicating progressive time points at which
25 the amount released or retained is measured. A corresponding family of rate release curves is thus generated, with each curve of the family illustrating the release characteristics of its corresponding slow:fast neutral lipid molar ratio when used with the aqueous
30 phase being tested.

The skilled practitioner can then select the formulation having the most desirable release characteristics for the particular therapeutic

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application of interest to obtain the desired control over release of the substance of interest (i.e., a biologically active compound) so as to deliver a therapeutically effective amount of the active compound to the individual to be administered the MVL formulation. A skilled practitioner can thus select a MVL formulation, in particular one having the most advantageous slow:fast neutral lipid molar ratio, for delivering a therapeutically effective dose over the optimum period of time so as to maximize the therapeutic effect of the drug or other biologically active compound administered during therapy.

For instance, if it is desired to produce a MVL formulation that releases a particular active compound *in vivo* in a relatively short period of time, i.e., over several hours after administration, the neutral lipid component that yields a release curve indicating such delivery characteristics when stored *in vitro* in plasma, or a plasma-like composition, at about 37°C will be selected. The proportion of the fast release neutral lipid in the ratio will be comparatively large in this circumstance. On the other hand, when it is desired to produce a MVL formulation that releases its active compound *in vivo* over a relatively long period of time, i.e., over tens of hours after administration, even up to 200 hours post administration, the neutral lipid component that yields a release curve indicating such delivery characteristics when incubated under *in vivo* conditions will be selected. In this case the proportion of the slow release neutral lipid in the molar ratio will be comparatively large, and in some instances the neutral lipid component will contain no fast release neutral lipid at all.

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The shelf-life stability of the formulations should also be determined by incubation of the formulations in the contemplated storage medium at whatever storage temperature is required to assure
5 integrity of the biologically active compound for a suitable period of time. For convenience, the self-life stability tests can also be conducted in plasma or a plasma-like medium, but one skilled in the art will be able to substitute a different suitable storage medium,
10 such as normal saline, for use with the biologically active compound of interest, if desired. Since many biologically active compounds require storage at temperatures in the range from about 2 to 8°C, it is recommended that the shelf-life stability tests be
15 conducted at a temperature in this range.

These procedures are illustrated in the Examples of this application. For instance, in formulations containing mixtures of triolein and tricaprylin, when the neutral lipid component was held constant and incremental
20 increases in the ratio of triolein to tricaprylin were made, MVL formulations characterized by increasingly slower release were obtained, as is illustrated in Example 14 (Figure 18). In Example 13, a graded family of formulations encapsulating sucrose and lysine-HCl and
25 containing mixtures of tripalmitolein and tricaprylin were prepared. A graded family of formulations was created with tripalmitolein:tricaprylin molar ratios of 0:1, 1:0, 1:9, 1:4, 1:2, and 1:1 by holding constant the amount of tripalmitolein and making incremental increases
30 in the amount of tricaprylin. In this Example, increasingly more rapid release was obtained for each incremental increase in the proportion of tricaprylin in the neutral lipid component.

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It should particularly be noted that, unlike most other types of liposomes, accurate *in vivo* release characteristics regarding MVL formulations cannot be predicted from *in vitro* release studies conducted in saline. For instance, MVL formulated with tricaproin, a triglyceride having only 6 carbons in its acyl moieties, as the only neutral lipid were not stable under *in vivo* conditions (in solutions containing plasma or serum albumin at 37°C, but were stable under storage conditions (in saline at 2-8°C for up to a week). However, formulations containing tricaproin:triolein molar ratios of 4:1, 9:1, and 18:1 were stable under *in vivo* conditions for at least 4 days, and yielded a graded set of release rate curves (Figure 14).

In another embodiment, the present invention provides liposomal compositions comprising a therapeutically effective amount of a biologically active compound encapsulated in a multivesicular liposome formulation wherein the formulation comprises a neutral lipid component with a molar ratio of slow release neutral lipid to fast release neutral lipid in the molar ratio range from about 0:1 to 1:0, for example 1:1 to 1:100 and generally from about 4:1 to 27:1. The molar ratio of the neutral lipid component to the sum of the lipids in the MVL formulation is generally in the range from about 0.01 to about 0.21.

The preferred amphipathic lipids for use in making the multivesicular liposomes are phospholipids with even numbers of carbons in the carbon chain because such phospholipids are natural lipids found in the body and do not produce toxic metabolites. A representative list of amphipathic lipids preferred for use in the practice of this invention follows. Also included are abbreviations

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that may be used to refer to particular phospholipids in this application.

DOPC or DC18:1PC = 1,2-dioleoyl-*sn*-glycero-3-phosphocholine

DLPC or DC12:0PC = 1,2-dilauroyl-*sn*-glycero-3-phosphocholine

5 DMPC or DC14:0PC = 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine

DPPC or DC16:0PC = 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine

DSPC or DC18:0PC = 1,2-distearoyl-*sn*-glycero-3-phosphocholine

DAPC or DC20:0PC =

1,2-diarachidoyl-*sn*-glycero-3-phosphocholine

10 DBPC or DC22:0PC = 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine

DC16:1PC = 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine

DC20:1PC = 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine

DEPC or DC22:1PC = 1,2-dierucoyl-*sn*-glycero-3-phosphocholine

DPPG = 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol

15 DOPG = 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol

The term "biologically active compound" as used herein means a chemical compound that is known in the art as having utility for modulating biological processes so as to achieve a desired effect in modulation or treatment of
20 an undesired existing condition in a living being, such as a medical, agricultural or cosmetic effect. Thus, biologically active compounds are generally selected from the broad categories of medicaments, pharmaceuticals, radioisotopes, agricultural products, and cosmetics.

25 Therapeutic biologically active compounds, or drugs for encapsulation in the methods and compositions of this invention include anti-neoplastic agents, anti-infective agents, hormones, anti-depressives, antiviral agents, anti-nociceptive agents, anxiolytics and
30 biologics.

Representative examples of anti-neoplastic agents useful in the compositions and methods of the present

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invention include methotrexate, taxol, tumor necrosis factor, chlorambucil, interleukins, etoposide, cytarabine, fluorouracil and vinblastine.

Representative examples of anti-infective agents
5 useful in the compositions and methods of the present invention include pentamidine, metronidazole, penicillin, cephalexin, tetracyclin and chloramphenicol.

Representative examples of anti-viral agents
useful in the composition and methods of the present
10 invention include dideoxycytidine, zidovudine, acyclovir, interferons, dideoxyinosine and ganciclovir.

Representative examples of anxiolytics and sedatives useful in the compositions and methods of the invention include benzodiazepines such as diazepam,
15 barbiturates such as phenobarbital and other compounds such as buspirone and haloperidol.

Representative examples of hormones useful in the compositions and methods of the present invention include estradiol, prednisone, insulin, growth hormone,
20 erythropoietin, and prostaglandins.

Representative examples of anti-depressives useful in the compositions and methods of the present invention include fluoxetine, trazodone, imipramine, and doxepin.

Representative examples of anti-nociceptives
25 useful in the compositions and methods of the present invention include hydromorphone, oxycodone, fentanyl, morphine and meperidine.

The term "biologics" encompasses nucleic acids (DNA and RNA), proteins and peptides, and includes
30 compounds such as cytokines, hormones (pituitary and hypophyseal hormones), growth factors, vaccines etc. Of particular interest are interleukin-2, insulin-like growth factor-1, interferons, insulin, heparin,

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leuprolide, granulocyte colony stimulating factor (GCSF), granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor, inhibin, tumor growth factor alpha and beta, Mullerian inhibitory substance,
5 calcitonin, and hepatitis B vaccine.

The biologically active compound can be employed in the present invention in various forms, such as molecular complexes or biologically acceptable salts. Representative examples of such salts are succinate,
10 hydrochloride, hydrobromide, sulfate, phosphate, nitrate, borate, acetate, maleate, tartrate, salicylate, metal salts (e.g., alkali or alkaline earth), ammonium or amine salts (e.g., quaternary ammonium), and the like. Furthermore, derivatives of the active substances such as
15 esters, amides, and ethers which have desirable retention and release characteristics, but which are readily hydrolyzed *in vivo* by physiological pH or enzymes, can also be employed.

As used herein the term "therapeutically effective
20 amount" means the amount of a biologically active compound necessary to induce a desired pharmacological effect. The amount can vary greatly according to the effectiveness of a particular active substance, the age, weight, and response of the individual host as well as
25 the nature and severity of the host's symptoms. Accordingly, there is no upper or lower critical limitation upon the amount of the active substance. The therapeutically effective amount to be employed in the present invention can readily be determined by those
30 skilled in the art.

It is believed that the neutral lipid component in MVL, which is unique to MVL among liposomal formulations, interacts with the *in vivo* environment in such a way as

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to affect the rate at which compounds encapsulated within the MVL are released. In particular, MVL having a neutral lipid component comprised of triglycerides with less than 6 carbons in the acyl moiety interact with the
5 *in vivo* environment so as to become completely destabilized virtually upon contact with blood plasma. For this reason, saline solutions do not accurately mimic the effect of the *in vivo* environment on the drug release characteristics of MVL, but it has been discovered that
10 *in vitro* release studies conducted using blood plasma or a plasma-like medium can be used to accurately determine the *in vivo* release characteristics of an MVL formulation.

The following examples illustrate the manner in
15 which the invention can be practiced. It is understood, however, that the examples are for the purpose of illustration, and the invention is not to be regarded as limited to any of the specific materials or conditions therein.

20

Example 1

G-CSF-containing MVL

1. Manufacture

For manufacture of multivesicular liposomes (MVL) containing granulocyte colony stimulating factor (G-CSF),
25 the lipid combination solution contained (per ml chloroform): 11 mg DOPC, 2.3 mg DPPG, 8.7 mg cholesterol and either 2.4 mg (2.7 umol) triolein or 1.3 mg (2.7 umol) tricaprylin. Lipid solutions which contained four different molar ratios of the neutral lipids triolein and
30 tricaprylin were prepared by mixing appropriate volumes of the triolein and tricaprylin containing lipid

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solutions. The molar ratios of triolein to tricaprylin were 100:0, 50:50, 25:75, 10:90, and 0:100.

The first aqueous phase solution for MVL formulations contained 174 mM glycine, 100 mM HCl, 0.001% Tween80™, and 100 ug/ml G-CSF, 1 ml was added to a vial containing 1 ml of the lipid combination and emulsified by fixing the capped vial in a horizontal configuration to the head of a vortex mixer (Scientific Products) and shaking at maximum speed (2400 oscillations/min.) for 6 minutes.

The final emulsion (2 ml) was divided and transferred to two vials containing 2.5ml 3.2% glucose and 40 mM lysine. The emulsion was dispersed into microscopic droplets by fixing the capped vial in a horizontal configuration to the head of a vortex mixer and shaking for 3 seconds at 2400 oscillations/min. The contents of the vial were transferred to a flask containing 5 ml of 3.2% glucose, 40 mM lysine. The chloroform was removed from the microscopic droplets or spherules by transferring the flask to a 37°C gyratory water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 10 - 15 cfh for 10 minutes. Multivesicular liposomes in suspension containing encapsulated G-CSF were obtained.

The particle suspensions were diluted 1:4 with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes. The supernatant solution was removed by aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation. The final washed product was resuspended at 25% packed-particle volume per total volume and stored at 2-8°C for subsequent studies.

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2. In vitro release rate

The *in vitro* release rate of the MVL prepared as described above was determined by incubation in saline solutions containing 70% citrated-human plasma and 0.1% sodium azide at 37°C. G-CSF was determined in the centrifuge-collected particle fraction by solubilization of samples in 50% IPA and quantitation by high pressure liquid chromatography and UV detection using known methods.

10 The dependence of the *in vitro* release rate of G-CSF on the composition of the neutral lipid component used in manufacture of the multivesicular liposomes is shown in Figure 1. As the triolein to tricaprylin molar ratio was increased, the rate of release of G-CSF from 15 the multivesicular liposomes decreased.

3. In vivo release rate

The triolein and tricaprylin MVL formulations of G-CSF were evaluated in a pharmacodynamic model in Syrian Golden Hamsters. Exogenous G-CSF stimulates neutrophil (granulocyte) production which can be evaluated by assaying blood samples for an increase in peripheral blood neutrophil number and correspondingly an increase in peripheral blood leukocyte number.

Therefore, the formulations containing tricaprylin 25 or triolein were evaluated for release in a pharmacodynamic hamster model that measured peak and duration of excess leukocyte (granulocyte) production caused by rhu G-CSF (Cohen et al. 1987, *Proc. Natl. Acad. Sci. USA*, 84:2484-2488). In these studies the 30 subcutaneous injection of a solution of G-CSF was used as a control for bioequivalence of the encapsulated protein. The rapid-release formulation containing tricaprylin was

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also used as a control for bioequivalence of the encapsulated protein. First, the peak and duration of granulocyte increase upon subcutaneous injection of hamsters with formulations containing all tricaprylin
5 (slow:fast molar ratio of 0:100) was compared against that caused by a solution of unencapsulated G-CSF and the longer-release duration triolein formulation (slow:fast mole ratio of 100:0). For each formulation, 3 to 5 hamsters received 75-100 ug G-CSF per kg. The results
10 (Figure 2), are as predicted by the *in vitro* release studies. The tricaprylin formulation provided a rapid release of G-CSF and stimulated granulocyte production similar to that of the unencapsulated G-CSF solution. The triolein formulation had a lower (and later) peak and
15 longer duration.

As a further control, a group of hamsters were injected with the triolein formulation solubilized with detergent (Tween 20™) immediately prior to injection. The granulocyte number found at 24 hours for the
20 solubilized formulation was similar to that observed resulting from injections of the G-CSF-solutions or the formulation containing only tricaprylin as the neutral lipid (Figure 2).

Example 2

25 1. Manufacture

Multivesicular liposomes containing granulocyte/macrophage-colony stimulating factor (GM-CSF) were manufactured as described in Example 1, but using neutral lipid molar ratios of triolein to tricaprylin of 100:0,
30 25:75 and 10:90.

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2. In vitro release in human plasma at 37°C

The suspensions were prepared and incubated in human plasma at 37°C as described in Example 1. GM-CSF remaining encapsulated was determined by solubilization of the particle fraction in 50% IPA and quantitation by high pressure liquid chromatography and UV detection using known methods.

The results of the *in vitro* release assay (Figure 3) demonstrate that the graded replacement of triolein with tricaprylin results in graded increases in release rate.

3. In vitro pharmacokinetics

BALBc mice (aged 7 to 8 weeks and weighing approximately 20 grams) were administered subcutaneous injections of liposomes containing GM-CSF manufactured with either 100% triolein or a 25:75 ratio of triolein to tricaprylin. Blood samples were collected before the injection and at 1, 2, 4, and 7 days post injection, and the plasma was assayed for GM-CSF concentration using an ELISA kit. The formulation manufactured with 25:75 triolein to tricaprylin provided a higher peak level and a shorter duration of detectable rhu GM-CSF compared to the formulation manufactured with 100% triolein as the neutral lipid (Figure 4). These results are as predicted by the *in vitro* assay in human plasma at 37°C, and show that the rate of release of encapsulated GM-CSF is increased by addition of tricaprylin to the neutral lipid component.

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Example 3

Depo/IGF-1

1. Manufacture

Multivesicular liposomes were manufactured with
5 rhu IGF (recombinant human insulin-like growth factor),
with ¹⁴C-sucrose as an osmotic spacer, and ammonium
citrate as a buffer. The lipid combination solution
contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g
cholesterol, and the triglyceride component was either
10 2.1 g triolein, 1.9 g tripalmitolein, 1.5 g trilaurin,
1.3 g tricaprln, or 1.1 g tricaprylin (molar ratio,
0.34:0.07:0.52:0.06).

The first aqueous phase solution contained (per
ml) 16 mg rhu IGF-1 (Chiron), 7% sucrose, and 20 mM
15 ammonium citrate, pH 5. A first emulsion was made by
high-speed mixing of 5 ml of the lipid combination
solution with 5 ml of the aqueous phase solution at 9000
rpm for 9 minutes at 25-27°C. The emulsion was sheared
into microdroplets (spherules) by addition of 30 ml 40 mM
20 lysine, 4% glucose solution to the mixing vessel and
mixing at 6000 rpm for 1 min. The chloroform was removed
from the microscopic droplets or spherules by
transferring the suspension to a flask containing 70 ml
of 40 mM lysine, 3.2% glucose solution, placing the flask
25 in 37°C gyrorotary water bath, and flushing the surface
of the suspension with nitrogen gas at a flow rate of 70
cfh for 20 minutes to obtain the MVL particles in
suspension.

The suspensions were diluted 1:4 with normal
30 saline, and the particles were harvested by
centrifugation at 800 X g for 10 minutes at room
temperature. The supernatant solution was removed by

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aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation.

2. In vitro release rates

5 The final washed product was resuspended at 25% packed-particle volume per total volume and stored at a temperature of 2-8, 25, 32, or 37°C for either 24 or 48 hours. The pellet fraction from the 25,000 X G X 2 minute centrifugation was solubilized and assayed by HPLC
10 for IGF-1 retained. Also, each of the IGF-1-containing formulations stored at 2-8°C was evaluated within 24 hours in the *in vitro* release assay described in Example 1 and the results are summarized in Figure 5. The formulations manufactured with triolein (C18:1) or
15 tripalmitolein (C16:1) showed a similar release rate profile. Greater than 70% of the encapsulated IGF-1 released in 7 days. The formulation manufactured with tricaprylin (C8) demonstrated a rapid release rate profile with nearly complete release of IGF-1 in 2 days.
20 The formulations manufactured with tricaprin (C10) or trilaurin (C12) released IGF-1 markedly more slowly than the standard triolein formulation, less than 50% released in 7 days. These results suggest that the acyl chain length of the triglyceride is not directly correlated
25 with the rate of release of encapsulated IGF-1 *in vitro*, because the release of the tricaprylin (C8) formulations was more rapid than that of the tricaprin (C10) or trilaurin (C12) formulations.

 It should be noted that storage of the trilaurin
30 formulations in saline for only a few days at 2-8°C resulted in morphological reorganization of the particles and an accompanying complete release of encapsulated

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materials. This effect would not be expected from the stability exhibited in the *in vitro* release assay at higher temperatures. This catastrophic effect was related to storage of the formulation at temperatures significantly lower than the freezing point of the neutral lipid. This effect is demonstrated in Examples 16 and 17 below.

3. *In vivo* pharmacokinetics

Male, Sprague-Dawley rats (weighing 250 to 300g) were given subcutaneous injections of the multivesicular liposome formulation containing IGF-1 that were manufactured with either tricaprylin or tripalmitolein as the neutral lipid. The tricaprylin formulation demonstrated a higher peak level of plasma rhu IGF-1 and a short release duration compared to the formulation manufactured with tripalmitolein (Figure 6). This result confirms the prediction of rapid release of multivesicular liposomes manufactured with tricaprylin based upon results of the *in vitro* release assay.

20

Example 4

1. Manufacture

For manufacture of multivesicular liposomes containing rhu-insulin, the lipid combination solution contained (per ml chloroform): 11 mg DOPC, 2.3 mg DPPG, 8.7 mg cholesterol and either 2.4 mg (2.7 μ mol) triolein or 1.3 mg (2.7 μ mol) tricaprylin as indicated.

The first aqueous phase solution formulations contained 7.5% 14 C-sucrose, 20 mM citric acid, 50 mM HCl and 5 mg/ml rhu-Insulin (E. Coli, Sigma Chemical Co., St. Louis, MO). A first emulsion was made by high-speed

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mixing of 4 ml of the lipid combination solution with 4 ml of the aqueous phase solution at 9000 rpm for 8 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by addition of a solution to
5 the mixing vessel of 20 ml 20 mM lysine, 4% glucose, and mixing at 3000 rpm for 1 min. The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 30 ml of a solution of 20 mM lysine, 4% glucose at 37°C in a
10 gyrorotary water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cubic feet per hour for 20 minutes to obtain the MVL particles in suspension.

The suspensions were diluted 1:4 with normal
15 saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes. The supernatant solution was removed by aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation. The final
20 washed product was resuspended at 25% packed-particle volume per total volume and stored at 2-8°C for subsequent studies.

2. In vitro release rates

The "in vitro" release assay was performed by
25 diluting 1 volume of stored suspension with 3.2 volumes of citrated, human plasma, and placing 0.6 ml of this suspension into microfuge tubes which were stoppered and incubated under dynamic conditions at 37°C. After 1, 3, 6, and 7 days, the tubes were centrifuged at 14,000 X g X
30 2 minutes, and the supernatant solution was transferred to another tube for bioassay. The pellet fractions of centrifuged samples were incubated in 1% Nonidet® NP-40

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(CalBiochem, San Diego, CA), 50 mM trifluoroacetic acid for 10 minutes at 37°C. The ¹⁴C sucrose and insulin retained by the pellet fraction was determined, respectively, by scintillation counting and reverse phase
5 HPLC using known methods. The results of these studies showed that the multivesicular liposomes manufactured with triolein as the neutral lipid released both sucrose and insulin in an equivalent and linear fashion, with nearly complete release in 7 days. By contrast, the
10 formulation manufactured with tricaprylin retained only 20 to 25% of the encapsulated sucrose and insulin after only 1 day of incubation in plasma (Figure 7).

Example 5

1. Manufacture

15 For manufacture of multivesicular liposomes containing morphine, a GMP-validatable, scalable process was used. The neutral lipid component molar ratios of triolein to tricaprylin were 1:4 or 1:9. Control formulations contained triolein or tricaprylin as the
20 sole neutral lipid. The lipid combination solution contained (per liter) 10.2 g DOPC, 2.0 g DPPG, 7.6 g cholesterol, 2.1 g to 1.1 g triglyceride depending on the molar ratio of triolein:tricaprylin, (molar ratio, 0.34:0.07:0.52:0.06).

25 The first aqueous phase solution contained (per liter) 21 g morphine sulfate pentahydrate, 0.01 N hydrochloric acid. A first emulsion was made by high-speed mixing of 0.62 l of lipid combination solution with 0.9 l of aqueous phase solution at 8000 rpm for 30
30 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by transferring to a second

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mixing vessel containing 15 liter 4 mM Lysine, 5.9% glucose solution; and mixing at 1250-1300 rpm for 1.5 min at 45°C. Chloroform was removed from spherules by sparging the suspension for 50 min at 45°C in stepped intervals: 17 min at 15 l/min, 5 min at 40 l/min, 28 min at 10 l/min. The MVL particles were thus obtained in suspension. The particles in the final suspension were concentrated and washed free of unencapsulated morphine by either cross-flow- or dia-filtration with 25 l of normal saline solution. The final washed product was stored at 2-8°C for subsequent studies.

2. In vitro release profiles

The *in vitro* release assays were performed by a 1:9 dilution of suspensions which contained 8 to 15 mg of encapsulated morphine sulfate per ml into human plasma. The suspensions were incubated at 37°C under dynamic conditions. After 1, 2, 4, and 7 days, samples were diluted 1:4 with normal saline, the particles were sedimented by centrifugation at 800 X g X 10 min and the particle fraction was assayed to determine the amount of morphine retained by solubilization of the pellet fraction with 50% IPA and assay by UV spectrophotometry using known methods.

Figure 8 shows the release rate profile of morphine release for these formulations into human plasma. As shown in earlier examples, as the ratio of the triolein to tricaprylin increases, the rate and extent of morphine release decreases.

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3. In vivo pharmacokinetics

The *in vivo* release of the multivesicular liposomes containing morphine manufactured with triolein and tricaprylin alone or in combination was evaluated in the Beagle dog using an epidural injection, pharmacokinetic model. In these studies the level of morphine released from the liposomal formulation injected at a epidural site was determined in the plasma and at an adjacent intrathecal site (CSF, cerebral spinal fluid) separated from the epidural site by the spinal meningeal membrane. Only the CSF results are shown (Figure 9).

A correlation is found between the *in vitro* release results summarized in Figure 8 and the release rate profiles observed in the dog model (Figure 9). The *in vivo* tricaprylin-containing formulation releases very rapidly, with nearly complete release in 24 hours. The Mean Residence Time (MRT) for the 100% tricaprylin formulation was similar to that for injection of unencapsulated morphine, i.e., 2.3 h vs. 1.6 h. The inclusion of a small amount of triolein during manufacturing, i.e., 1:9 or 1:4 triolein:tricaprylin molar ratio slows the release rate, extending the release duration over 4 to 5 days, with MRTs of 13.2 h and 15.3 h, respectively. The tricaprylin containing formulations all release more rapidly than the formulation using triolein as the only neutral lipid, which had a MRT of 69 h in this model.

4. Storage Stability

The step-wise alteration in the neutral lipid composition of the MVL to provide a family of formulations with step-wise increase in the release rate profiles of bioactive compound does not seem to

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compromise the storage stability of the formulations provided that the formulations are stored near or above the melting point of the neutral lipid combination. Batches of the 10:90 triolein:tricaprylin morphine MVL
5 formulation, described above and which release rapidly both *in vitro* and *in vivo*, were evaluated for dependence of the release rate on temperature of storage. The MVL suspensions in saline storage solution were incubated at the normal storage temperature, 2-8°C (4°C nominal) and at
10 elevated temperatures, 26, 37, and 41°C. The results of these studies are shown in Figure 10, an Arrhenius plot, which plots the logarithm of the release rate against 1/Temperature (°K). The slope of the plot appears to be continuous. The release rate profile at high temperature
15 supports the observation that the MVL stored in saline solution at 2-8°C have a very slow release (about 1% of the encapsulated morphine per 100 days) and therefore can be expected to have a shelf-life in excess of a year if a criteria of 5 or 10% release of encapsulated material is
20 the limit for shelf-life. Further, it is also evident that a physiological matrix such as plasma (Figure 9) or the *in vivo* environment of the epidural space (Figure 9) greatly accelerates the release when compared to the release in saline solution at 37°C, i.e., 50% of the
25 encapsulated morphine released per day, versus less than 1% of encapsulated morphine per day in the latter.

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Example 6

Cytosine Arabinoside

1. Manufacture

Multivesicular liposomes containing cytosine
5 arabinoside (AraC) were manufactured with various
substitutions for the phosphatidylcholine component of
the lipid organic phase solution. The substitutions were
performed to determine the effect on the release rate
profile of changing the acyl component in the major
10 phospholipid in a MVL formulation containing either
triolein or tricaprylin as the neutral lipid.

In this first example, DOPC is used to demonstrate
that the release rate in a physiological medium from MVL
formulations containing AraC/HCl in the aqueous phase can
15 be modified by adjusting the triolein to tricaprylin
ratio. The lipid combinations contained (per 1200 ml)
122.4 ml DOPC at 100 mg/ml, 2.4 g DPPG, 9.12 g
cholesterol, 2.5 g triolein or 1.3 g tricaprylin, or a
mixture thereof yielding a molar ratio of triolein to
20 tricaprylin of 1:4, 1:9, 1:18, or 1:27.

The first aqueous phase solution contained 20
mg/ml cytosine arabinoside, 0.1 N HCl. A first emulsion
was made by high speed mixing of 10 ml lipid combination
and 10 ml of first aqueous phase solution at 9000 rpm for
25 9 min. The emulsion was sheared into microdroplets by
transfer of first emulsion to second mixing vessel
containing 200 ml of 3.2% glucose, 40 mM lysine and
mixing at 2100 rpm for 2.5 minutes. The chloroform was
removed by transferring suspension to two, 1-liter flasks
30 and flushing at 70 cfh. The final suspension was diluted
1:2 with saline and particles collected by centrifugation

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at 800 X g X 10 min washed 3 times, resuspended to 33% lipocrit, and then adjusted to 10 mg/ml.

2. In vitro release into human plasma

The *in vitro* release rate profiles of the cytosine arabinoside (AraC) MVL formulations were obtained by dilution of the suspension 1:9 into human plasma and incubation of samples at 37°C under dynamic conditions. At time points of 1, 2, 4, and 7 days, 1.2 ml of saline was added to triplicate 0.3 ml samples, and the particle fraction was collected by centrifugation at 16,000 X g X 2 min. The supernatant fraction was removed by aspiration, and the particle fraction was resuspended in 1 ml of 50% isopropyl alcohol, vortexed, incubated at 37°C for 10 min, centrifuged and then 0.06 or 0.2 ml of the supernatant was added to 1.0 ml of 0.1 N HCl. Cytosine arabinoside was determined by U.V. samples at 280 nm.

The *in vitro* release profiles measuring AraC retained by the formulations of multivesicular liposomes are shown in Figure 11. Release from the formulation having only tricaprylin as the neutral lipid was rapid. As the ratio of tricaprylin to triolein used in the manufacture of the MVL formulation increases, the rate of release of cytosine arabinoside by the MVL incubated in plasma decreases. Thus, a family of release rate curves with predictably increasing release rates is created by incremental increases in the amount of triolein in the triolein to tricaprylin ratio.

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Example 7

Effect of Substitution of Higher Transition
Temperature PhosphatidylCholines or Longer Chain Length
for DOPC in Formulations Containing Tricaprylin

5 The ranking in a family of *in vitro* release rate
profiles (as obtained in Example 6) with the greater
molar ratio of tricaprylin to triolein resulting in
faster release both *in vivo* and in *in vitro* models
(plasma at 37°C), remains consistent when a
10 phosphatidylcholine of higher phase transition
temperature or of increased chain length is substituted
for DOPC in the formulations of Example 6. In this
example distearoylphosphatidylcholine (DSPC, mp 55°C) was
substituted for DOPC (mp 0°C).

15 1. Manufacture

 The manufacturing parameters were adjusted to
accommodate the higher mp of DSPC by performing
emulsification at 50 °C. The manufacture of emulsions
used for the DOPC control formulations was performed at
20 ambient temperature. In addition, the HCl concentration
of the first aqueous phase solution was increased 36%
over that in Example 6.

 The lipid combination solution contained (per
liter) 10.2 g DOPC or 10.3 g DSPC, 2.1 g DPPG, 7.7 g
25 cholesterol, and the triglyceride component was either
1.1 g tricaprylin or 2.1 g triolein (molar ratio of
0.34:0.07:0.52:0.06). Mixtures of triolein and
tricaprylin were blended to provide the ratios of 1:4,
1:9, 1:18, 1:2 triolein to tricaprylin for use in the
30 formulations.

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The first aqueous phase contained (per ml) 20 mg cytosine arabinoside and 136 mM Hcl. An emulsion was made by high-speed mixing of 10 ml of the lipid combination solution with 10 ml of the aqueous phase solution using a 2 cm diameter blade in a stainless steel vessel at 9000 rpm for 8 minutes at room temperature (for formulations with DOPC) or 55°C (for formulations with DSPC). The emulsion was sheared into microdroplets by transfer into a 400 ml glass Mason jar containing 200 ml of 5% glucose and 40 mM lysine and, using a 4 cm diameter blade, mixing at 4000 rpm for 1.5 minutes at the same temperature as used for the first emulsification. Chloroform was removed by placing the container in a 37°C gyrorotary water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes.

The suspensions were diluted 1:4 with normal saline and the particles were collected by centrifugation at 800 x g for 10 minutes at room temperature. The supernatant was removed by aspiration, and then the particles were washed twice by resuspension in normal saline solution and centrifugation. The final washed pellet was resuspended in normal saline solution and adjusted to 10 mg/ml of cytosine arabinoside.

25 2. In vitro release profiles

The *in vitro* release assay of AraC from the MVLs was performed in human plasma as described in Example 6 above. As shown in Figure 12, in MVL in which the higher melting point DSPC was substituted for DOPC, a family of graded release rate profiles was obtained by varying the molar ratio of tricaprylin to triolein, with the faster release formulations containing a higher molar ratio of

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tricaprylin to triolein. As has been shown previously for other formulations of MVL, an increase in hydrochloric acid concentration in the aqueous phase solution (as compared with that contained in the MVL of Example 6) can slow the release of the active substance *in vitro*.

Example 8

Sucrose

1. Manufacture

10 In this example, the effect of tricaprylin on the release rate was tested for formulations containing 4% sucrose as the active agent in formulations having dieucrylphosphatidylcholine (DEPC, mp <0°C), a 22 carbon chain length phosphatidylcholine, substituted for
15 dioleolyphosphatidylcholine (DOPC, mp <0°C), a 18 carbon chain length phosphatidylcholine.

The lipid solution contained (per liter) either 10.2 g DOPC or 11.0 g DEPC and 2.1 g DPPG, 7.7 g cholesterol, and the triglyceride component was either
20 1.1 g tricaprylin or 2.1 g triolein (molar ratio of 0.34:0.07:0.52:0.06). For mixtures of the neutral lipid, triolein- and tricaprylin-containing lipid combinations were blended to provide these triolein to tricaprylin molar ratios.

25 The first aqueous phase contained 4% Sucrose (Spectrum USP/NF, Los Angeles, CA) and was spiked with 40 uL of ¹⁴C sucrose. A first emulsion was made by high-speed mixing of 5 ml of the lipid combination solution with 5 ml of the aqueous phase solution using a 2 cm
30 diameter blade in a stainless steel vessel at 9000 rpm for 10 minutes at room temperature. The emulsion was

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sheared into microdroplets by transfer to a 400 ml glass Mason jar containing 200 ml of 4% glucose and 4 mM lysine for 2 minutes, using a 4 cm diameter blade and mixing at 3500 rpm. Chloroform was removed from the droplets by
5 transferring the suspension to a 1 liter culture flask placed in 37°C gyratory water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes.

The particle suspensions were diluted 1:2 with
10 normal saline, and the particles were collected by centrifugation at 800 x g for 10 minutes at room temperature. The supernatant was removed by aspiration, and the particles were washed twice by resuspension in normal saline solution and centrifugation. The final,
15 washed particle pellet was resuspended in normal saline solution and adjusted to approximately 33% lipocrit.

2. In vitro plasma release studies

For *in vitro*, plasma release studies, the suspensions were diluted 1:10 in human plasma with 0.1%
20 sodium azide. Triplicate aliquots of 300 uL were incubated in 1.5 ml screw-top Eppendorf tubes and harvested on days 0, 1, 2, 3, and 4. Samples were harvested by pulling tubes from the incubator at random, labeling the tube with day of pull, diluting the contents
25 with 1.2 ml of normal saline solution, and centrifuging at 27,000 x g for 5 minutes in a microfuge. The supernatant was carefully aspirated away from the particle pellet. The pellet fraction was resuspended in
30 1 ml of 50% IPA by vortexing, incubating at 37°C for 10 minutes, and then vortexing. A 50 ul sample was then diluted with 3 ml of scintillation fluid in a

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scintillation vial, the vial was shaken vigorously and sucrose was determined by scintillation counting.

As can be seen from the data contained in Figure 13, the formulation containing only tricaprylin released all encapsulated sucrose within one day when incubated in plasma. The ratio of triolein: tricaprylin of 1:18 was required to achieve an intermediate rate of release for an MVL formulation with this aqueous phase when DEPC replaced DOPC.

10

Example 9

In this example, tricaproin (C6) was substituted for tricaprylin (C8) as a release rate modifying neutral lipid.

1. Manufacture

15 The lipid combination solution contained (per liter) a combination of 10.2 g, 2.1 g DPPG, 7.7 g cholesterol, and the triglyceride component was either 0.9 g tricaproin or 2.1 g triolein (molar ratio of 0.34:0.07:0.52:0.06). Mixtures of the lipid solutions containing triolein and tricaproin, were blended to provide lipid solutions containing molar ratios of triolein to caproin of 1:4, 1:9 and 1:18. These formulations were manufactured and tested as described in Example 8.

25 The first aqueous phase contained 4% Sucrose (Spectrum USP/NF) and was spiked with 40 uL of ¹⁴C Sucrose (ICN lot#54661027). A first emulsion was made by high-speed mixing of 5 ml of the lipid combination solution with 5 ml of the aqueous phase solution using a 2 cm diameter blade in a stainless steel vessel at 9000 rpm for 10 minutes at room temperature. The second

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emulsification was performed in a 400 ml glass Mason jar containing 200 ml of 4% glucose and 4 mM lysine, using a 4 cm diameter blade and mixing at 3500 rpm for 2 minutes. Chloroform was removed by transferring the contents of
5 the jar to a 1 liter culture flask placed in 37°C gyrorotary water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes.

The suspensions were diluted 1:2 with normal
10 saline, and the particles were harvested by centrifugation at 800 x g for 10 minutes at room temperature. The supernatant was removed by aspiration, and the particles were washed twice by resuspension in normal saline solution and centrifugation. The final
15 washed pellet was resuspended in normal saline solution and adjusted to approximately 33% lipocrit wherein lipocrit in percent is the volume occupied by the liposomes divided by the total volume of the liposome suspension multiplied by one hundred. The yield for each
20 variation in the neutral lipid was greater than 50%. Following manufacture the free sucrose concentration at 33% lipocrit was approximately 3% of the total sucrose concentration.

2. In vitro release profiles

25 For *in vitro* plasma release studies, the suspensions were diluted 1:10 in human plasma. Triplicate aliquots of 300 uL were incubated dynamically in 1.5 ml screw-top Eppendorf tubes and harvested on days 0,1,2,3, and 4. Samples were harvested by pulling tubes
30 from the incubator at random, labeling the tube with the day of the pull, diluting the contents with 1.2 ml of normal saline solution, and centrifuging at 27,000 x g

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for 5 minutes in microfuge. The supernatant was carefully aspirated away from the pellet. The pellet fraction was resuspended in 1 ml of 50% IPA by vortexing, incubating at 37°C for 10 minutes, and then vortexing
5 again. A 50 ul sample was then diluted with 3 ml of scintillation fluid in a scintillation vial, the vial was shaken vigorously, and ¹⁴C-sucrose radioactivity was determined by scintillation counting.

As shown in Figure 14, the substitution of
10 tricaproin for tricaprylin in mixtures containing triolein as a release rate modifying neutral lipid combination results in formulations having a graded family of release rates for various triolein:tricaproin molar ratios. The greater the molar ratio of tricaproin
15 to triolein, the more rapid the release of sucrose. There is a distinguishing difference between tricaproin and tricaprylin containing formations. With this aqueous phase, i.e., containing sucrose as the encapsulated active agent, the multivesicular particles manufactured
20 using only tricaproin underwent a physical transformation and released their contents within 5 minutes of dilution into human plasma at room temperature. Dilution into saline solution containing 0.5% bovine serum albumin had the same effect. By contrast, the formulations
25 containing only tricaprylin as the neutral lipid generally required 12 or more hours of incubation in plasma at 37°C for complete release of the active agent. Despite the instability of the tricaproin only formulations in human plasma or saline at room
30 temperature. The formulations were stable during storage at 2-8°C in saline for at least a week.

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Example 10

Antisense Oligonucleotides

This example illustrates use of the method of the invention to encapsulate antisense oligonucleotides.

5 1. Manufacture

The first aqueous phase solution contained (per ml) 5, 10 or 20 mg of an IL-6 antisense oligonucleotide (donated by Leu Neckers, NIH, Bethesda, MD), which in some studies was biotinylated, and 5% mannitol.

10 Hydrochloric acid (0.1 ml of 1 N) was added to a vial containing 1 ml of the lipid combination of Example 1 and the combination was emulsified by fixing the capped vial in a horizontal configuration to the head of a vortex mixer (Scientific Products) and shaking at 2400
15 oscillations/min for 1 minute. The remainder of the first aqueous phase solution (0.9 ml 5% mannitol containing 10 mg antisense oligonucleotide) was added to the vial, and emulsification was continued for 5 minutes.

The final emulsion (2 ml) was divided and
20 transferred to two vials containing 2.5 ml 3.2% glucose, and 40 mM lysine. The emulsion was dispersed into microscopic droplets by fixing the capped vial in a horizontal configuration to the head of a vortex mixer and shaking for 3 seconds at approximately 1200 rpm. The
25 contents of the vial were transferred to a flask containing 5 ml of 3.2% glucose, 40 mM lysine, and the chloroform was removed from the microscopic droplets or spherules by transferring the flask to a 37°C gyrorotary water bath, and flushing the surface of the suspension
30 with nitrogen gas at a flow rate of 15 cubic feet per hour for 10 minutes.

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The suspensions were diluted 1:4 with normal saline and the particles were harvested by centrifugation at 800 X g for 10 minutes. The supernatant solution was removed by aspiration, and the particles were washed
5 twice by resuspension in fresh, normal saline solution and centrifugation. The final washed product was resuspended at 25% packed-particle volume per total volume and stored at 2-8°C for subsequent studies.

The recovery of encapsulated oligonucleotide was
10 determined by diluting a sample of the suspension 1:1 with 0.1% SDS, incubating the sample in a boiling water bath for 2 minutes, and then diluting the sample (typically 1/20) into 0.1 N NaOH for determination of UV
absorption using a wavelength scan from 320 to 212
15 nmeters. The concentration of oligonucleotide in the sample was calculated by subtracting the measured A320 absorbance value from the A257 absorbance value. A sample of the first aqueous phase solution served as the standard, 17.6 (A257-1 cm) units per mg/ml.

20 2. In vitro release profiles

The *in vitro* release characteristics of multivesicular particles containing oligonucleotides were determined by measuring the amount of oligonucleotide remaining with particle fraction when incubated at 37°C
25 in rat cerebral spinal fluid (CSF). Samples stored in normal saline were resuspended in the storage solution, and centrifuged at 750 X g for 10 minutes. The saline supernatant solution was removed by aspiration, and the particles were resuspended in rat CSF to concentrations
30 of 0.25 to 0.5 mg of the oligonucleotide per ml of CSF. The samples were incubated at 37 °C under static conditions. After 0, 1, 2, 3, and 7 days, samples of the

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particle/CSF suspensions were removed, diluted 10-fold in saline, centrifuged, and the supernatant was aspirated away from the particle fraction. The particle fraction was incubated in 0.1% SDS and diluted with 0.1 N NaOH, and a UV absorbance spectrum of the oligonucleotide was obtained over 340 to 210 nmeter range. The amount of oligonucleotide retained by the particle fraction was determined as above.

As shown in Figure 15, the tricaprylin formulation did not release *in vitro* more rapidly, but rather had a greater overall release; the triolein-containing formulation stopped releasing the drug in rut CSF after about 2.5 days as compared with continued release by the tricaprylin-containing formulation.

3. *In vivo* pharmacokinetics

When the antisense MVL formulations manufactured with tricaprylin as the neutral lipid were tested *in vivo* by intrathecal injection in rats and samples of the CSF subsequently taken were examined microscopically, no particles were evident in CSF after two days. The MVL particles manufactured with triolein were evident at two days but had a "shrunk" appearance. There was no evidence of free, native oligonucleotide by a 3'-end labeling assay.

To improve the sensitivity of the *in vivo* assay for oligonucleotide concentration in CSF, the oligonucleotide was biotinylated and the biotinylated oligonucleotide was formulated as described above in MVL using either triolein or tricaprylin as the neutral lipid. The recoveries of encapsulated biotinylated oligonucleotide were 78% for triolein and 82% for tricaprylin-containing MVL. When studied *in vivo*,

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biotinylated oligonucleotide, found free in the CSF released from the tricaprylin-containing liposomes was below the limit of detection after 2 days. Free, biotinylated oligonucleotide was present in the rat sample CSF 4 days after administration of the triolein-containing formulations at a concentration of approximately 0.5 uM (results not shown). The *in vivo* data are consistent with the tricaprylin MVL rapidly releasing antisense oligonucleotide *in vivo*, and the triolein formulation providing a more sustained release of the oligonucleotides.

Example 11

Plasmid-containing MVL

1. Manufacture

For manufacture of multivesicular liposomes encapsulating the *E. coli* PBR322 plasmid, the lipid combination solution contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, 2.16 g triolein (mw 885.40) or 1.15 g tricaprylin (mw 470.7) (DOPC : DPPG : Cholesterol : triglyceride molar ratio, 0.34:0.07:0.52:0.06).

The first aqueous phase solution contained (per liter) 42 g ¹⁴C-sucrose (0.6 uCi per ml) 100 mmol lysine, 84 mmol hydrochloric acid, pH 7.4 and 20 ug/ml PBR322 plasmid (Promega, Madison WI). A first emulsion was made by high-speed mixing of 3 ml of lipid combination solution with 3 ml of aqueous phase solution at 9000 rpm for 9 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by addition of 20 ml of a 20 mM lysine, 4% glucose solution to the mixing vessel and further mixing at 4000 rpm for 2 min. The chloroform was

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removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 30 ml of 20 mM lysine, 4% glucose solution at 37°C in a gyrorotary water bath, and flushing the surface of the
5 suspension with nitrogen gas at a flow rate of 70 liter per hr. for 20 minutes to form the MVL.

The MVL suspensions were diluted 1:4 with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes. The
10 supernatant solution was removed by aspiration and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation. The final washed product was resuspended to 25% packed-particle volume per total volume, and stored at 2-8°C for
15 subsequent studies.

2. In vitro release profile

The *in vitro* release assays were performed by a 1:2.5 dilution of suspensions which contained the multivesicular liposomes encapsulating PBR322 plasmid and
20 ¹⁴C-sucrose into human plasma. Previous studies had established that ¹⁴C-sucrose release was an adequate surrogate for estimating the release of the PBR322 plasmid from the MVL. The suspensions were incubated at 37°C under static conditions. At times of 0, 1, 2, 3, 6,
25 10, and 17 days, samples were diluted 1:4 with normal saline, particles were sedimented by centrifugation at 800 X g X 10 min, and the particle fraction was assayed by dissolving samples in a scintillation counting solution and performing scintillation counting of the
30 amount of ¹⁴C-sucrose retained by the particle fraction.

The results of this study shown in Figure 16 indicate the PBR322 plasmid formulation using triolein as

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the neutral lipid was stable under simulated *in vivo* conditions and released about 30% of the encapsulated ¹⁴C-sucrose over a span of 17 days; whereas, the tricaprylin-formulated particles rapidly released their contents upon contact with human plasma at 37°C.

Example 12

In the following example sucrose and lysine-HCl used as excipients in the PBR322 plasmid formulation were encapsulated without additional active agent to yield an "excipient only" family of graded, sucrose release formulations using tripalmitolein:tricaprylin molar ratios as the neutral lipid.

1. Manufacture

For manufacture of multivesicular liposomes encapsulating only sucrose-lysine HCl, the lipid combination solution contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, 1.9 g to 1.0 g triglyceride depending on the molar ratio of tripalmitolein (mw 801, C16:1 9C):tricaprylin to yield a DOPC:DPPG:cholesterol:triglyceride molar ratio of 0.34:0.07:0.52:0.06. The molar ratios of tripalmitolein to tricaprylin prepared in the formulations of this example were 0:1, 1:0, 1:9, 1:4, 1:2, and 1:1.

The first aqueous phase solution contained (per liter) 42 g ¹⁴C-sucrose (1.0 µCi per ml), 100 mmol lysine, 90 mmol hydrochloric acid, pH 5.7. A first emulsion was made by high-speed mixing of 5 ml of lipid combination solution with 5 ml of aqueous phase solution at 9000 rpm for 9 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by addition of 20 ml of a 20 mM

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lysine, 4% glucose solution to the mixing vessel, and mixing at 5000 rpm for 1.5 min. The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 30 ml
5 of the 20 mM lysine, 4% glucose solution in a 37°C gyrorotary water bath, and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cubic feet per hr. for 20 minutes to obtain the multivesicular particles in suspension.

10 The suspensions were diluted 1:4 with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes. The supernatant solution was removed by aspiration and the particles were washed twice by resuspension in fresh,
15 normal saline solution and centrifugation. The final washed product was resuspended at 25% packed-particle volume per total volume and stored at 2-8°C for subsequent studies.

2. In vitro release profile

20 The "in vitro" release assays were performed by a 1:9 dilution into human plasma of suspensions which contained multivesicular liposome encapsulating ¹⁴C-sucrose. The suspensions were incubated at 37°C under dynamic gentle mixing. At time points of 0, 1, 3, 5, 8,
25 and 12 days, samples were diluted 1:4 with normal saline, the particles were sedimented by centrifugation at 800 X g for 10 min, and the particle fraction was assayed by dissolving in scintillation counting solution and scintillation counting of the amount of ¹⁴C-sucrose
30 retained in the particles. The results of these studies (Figure 17) show that a 1:1 molar ratio of tripalmitolein to tricaprylin provided a somewhat slower release rate

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than tripalmitolein alone. A graded family of release rates was obtained having faster release by increasing the proportion of tricaprylin in the neutral lipid component.

5

Example 13

Anesthetics

1. **Tetracaine MVL formulations**

For manufacture of MVL encapsulating tetracaine, the lipid combination solution contained (per ml
10 chloroform) 15.6 mg DOPC, 3.1 mg DPPG, 11.5 mg cholesterol, and 12.6 mg to 6.6 mg triglyceride depending on the molar ratio of triolein:tricaprylin. The lipophilicity of tetracaine was found to require that the concentration of lipid (68 $\mu\text{mol/ml}$) in the lipid
15 combination be increased 2 to 1.5 times higher to obtain satisfactory MVL formulations. The triglyceride was enriched as well; the molar ratio of DOPC:DPPG:cholesterol:triglyceride was 0.29 : 0.06 : 0.44 : 0.21. The molar ratios of triolein to tricaprylin
20 prepared in the formulations of this example were 1:0; 1:10; 0.5:10; 0.2:10; 0.1:10; 0.05:10; and 0:1. The first aqueous phase solution for encapsulation of tetracaine contained (per ml) 15 mg of tetracaine phosphate and 200 mg of alpha-cyclodextrin polymer.

25 An aliquot of first aqueous phase solution (1 ml) was added to a vial containing 1 ml of the lipid combination and emulsified by fixing the capped vial in a horizontal configuration to the head of a vortex mixer (Scientific Products), and shaking at 2400
30 oscillations/min for 12 minutes.

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The final emulsion (1 ml) was divided and transferred to two vials containing 2.5 ml of a solution of 3.2% glucose, 5 mM lysine. The emulsion was dispersed into microscopic droplets by fixing the capped vial in a horizontal configuration to the head of a vortex mixer and shaking for 3 seconds at a setting of 600-800 oscillations/min. The contents of the vial were transferred to a flask containing 50 ml of a solution of 3.2% glucose, 5 mM lysine, and the chloroform was removed from the microscopic droplets or spherules by transferring the flask to a 37°C gyrorotary water bath, and flushing the surface of the suspension with nitrogen gas at a flow rate of one liter per min. for 20 minutes.

The suspensions were diluted 1:4 by volume with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes. The supernatant solution was removed by aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation.

2. In vivo pharmacokinetics

The *in vivo* release characteristics of the multivesicular particles were determined in BalbC mice (aged 7 to 8 weeks; weighing approximately 20 grams) by subcutaneous injection in the abdomen region (100 ul). At time points of 0, 5, and 24 hours after injection, mice were sacrificed, the subcutaneous tissue was harvested, homogenized, and extracted, and extracts were assayed by HPLC using UV detection for the amount of tetracaine retained.

The desired *in vivo* release duration for the tetracaine MVL was 24 hours. Formulations which contained triolein only as the neutral lipid were found

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to be stable *in vivo* and released over too long a duration.

By including tricaprylin in the neutral lipid component, formulations with shorter duration and more desirable pharmacokinetic profiles were obtained (Figure 18). The desired 24 hour release duration for the anesthetic was provided by a formulation with a 1 triolein: 100 tricaprylin ratio.

**The Relationship Between Neutral Lipid
Selection and Intended Storage Temperature.**

As shown in the following examples, the melting point of the neutral lipid is an important consideration in selection of the rate-modifying neutral lipid. However, other factors must also be taken into account, for example, the composition of the first aqueous phase solution. The conclusion to these examples is that the freezing (melting or cloud) point of the neutral lipid or neutral lipid mixture should be above or near the storage temperature in order to assure storage stability. If formulations are stored at temperatures significantly lower than the freezing point of the neutral lipid or mixture thereof, the MVL particles undergo a physical, morphological transition, which results in loss of internal structure and release of encapsulated materials. This transition may occur within a few hours or over several days or weeks, depending on the composition of the first aqueous phase composition.

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Example 14

In the following example, MVLs were manufactured with either tricaprylin or tricaprin as the neutral lipid component. The freezing point of tricaprylin is 8°C and
5 that of tricaprin is 31 °C. The first aqueous phase contained either cytosine arabinoside or morphine sulfate in 0.1 N HCl. Aliquots of the final product were stored in saline at 2- 8, 25, 32, and 37 °C. The release of encapsulated materials was determined over the time of
10 storage.

1. Manufacture

For manufacture of MVLs, the lipid combination solution contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, and the triglyceride component was
15 either 0.93 g tricaprylin (C8) or 1.1 g tricaprin (C10) (molar ratio, 0.34:0.07:0.52:0.06). The first aqueous phase solution contained either (per ml) 20 mg cytosine arabinoside in 0.1 N HCl or 20 mg morphine sulfate pentahydrate in 0.1 N HCl.

20 For formulations encapsulating cytosine arabinoside, the first emulsion was made by high-speed mixing of 10 ml of lipid combination solution with 10 ml of aqueous phase solution at 9000 rpm for 14 minutes at 25-27°C with a high shear blade. For formulations
25 encapsulating morphine, the first emulsion was made by high-speed mixing of 12.5 ml of the lipid combination solution with 7.5 ml of the aqueous phase solution at 9000 rpm for 14 minutes at 25-27°C. The first emulsions were sheared into microdroplets (spherules) by transfer
30 to a mixing chamber containing 200 ml of 40 mM lysine, 3.2% glucose solution, and mixing at 2100 rpm for 2.5

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min. The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask, placing the flask in 37°C gyratory water bath, and flushing the surface of the suspension with nitrogen
5 gas at a flow rate of 70 cfh for 20 minutes.

The suspensions were diluted 1:4 by volume with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes at room temperature. The supernatant solution was removed by
10 aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation. The final washed product was resuspended at 33% packed-particle volume and stored.

The characterization of yields of encapsulated and
15 free (supernatant) cytosine arabinoside and morphine from the tricapylin and tricaprln MVL formulations was performed a few hours post-manufacture (Table 2). The yield of encapsulated cytosine arabinoside and morphine was acceptable, and the free (supernatant) concentrations
20 of cytosine arabinoside and morphine were low for both the tricapylin and tricaprln formulations.

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TABLE 2

	First Aqueous Phase Active Agent	Neutral Lipid	Yield, % of Initial Active	Storage Concen- tration, mg/ml	Supernatant Concen- tration, mg/ml
5	Cytosine	Tricaprylin	42	7.0	0.3
	Arabinoside	Tricaprin	37	6.2	0.2
	Morphine Sulfate	Tricaprylin	46	11.2	0.3
		Tricaprin	36	8.9	0.2

2. Storage release profiles

The suspensions were stored at 2-8, 25, 32 or
 10 37°C. At time points of 24, 48 and 200 hours the
 suspensions were centrifuged at 25,000 X G for 2 minutes,
 and supernatant solutions were analyzed for content of
 cytosine arabinoside or morphine by 1:1 volume dilution
 with 50% isopropyl alcohol, vortexing, incubation at 37°C
 15 for 10 min, and centrifugation. The supernatant samples
 were analyzed by dilution into 0.1 N HCl and the
 concentration of cytosine arabinoside was measured by
 absorbance determined at 280 nm, or dilution into 0.1 N
 NaOH, and the morphine concentration was measured by
 20 absorbance determined at 298 nm.

The results of these storage-temperature-effect
 studies are shown in Figures 19A-D. Release rates from
 the MVL containing tricaprylin (melting point 8°C) are
 very slow at 2-8°C. Release rate increased with
 25 increasing storage temperature as would be expected as
 consistent with acceleration of release with elevation of
 temperature (Figures 19A and 19C). The AraC-MVL
 containing tricaprin (melting point 31°C), however,

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released the active agent at a very fast rate when stored below the melting point of the triglyceride. With storage of the tricaprln MVL at higher temperatures 25° and 32°, the release rate decreased, but with storage at 5 37° again increased (Figure 19B). In fact, the tricaprln-containing AraC-MVL particles stored below the melting point temperature of the neutral lipid tricaprln (31°C), were completely destabilized and released the encapsulated morphine over a few hours (Figure 19B).

10 The results for the storage study of cytosine arabinoside-containing MVL are shown in Figure 20 by an Arrhenius plot. The tricaprln MVL formulation showed an expected continuous linear relationship when log of the release rate is plotted versus temperature, 15 suggesting that a single process is responsible for release. On the other hand, the plot of the data for the tricaprln formulation was discontinuous, indicating that two processes are responsible for release in the temperature range studied. At higher temperatures above 20 the freezing point of tricaprln, the slope was as expected, i.e., rates increased with increased temperature of storage. The second process, the melting point effect, is observed below the freezing point of tricaprln wherein, the apparent rate increased with 25 decreasing temperature.

 Again, comparison of the release rate profiles (19A-D) of particles having cytosine arabinoside encapsulated as a first aqueous phase component with particles having morphine sulfate encapsulated indicates 30 that onset of the destabilizing effect caused by storage of formulations below the melting point of the neutral lipid, called herein "the melting point effect," is dependent on the composition of the first aqueous phase

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solution. Onset of the melting point effect of tricaprin MVL during storage was observed to be rapid with cytosine arabinoside-containing formulations, (Figure 19B) but required several days for the morphine sulfate-containing formulations (Figure 19D). Further, the results of these studies suggest that the further below the freezing point the formulation is stored, the more rapid the onset of the melting point effect.

Example 15

10 In this example, the aqueous phase contained sucrose, cytosine arabinoside and 0.1 N HCl as an osmotic spacer. MVL were manufactured with triolein (mp 8°C), tricaprin (mp 31°C), or trilaurin (mp 46 °C) as the neutral lipid. The final product was stored at a
15 temperature of 2-8°C, 22, or 37°C, and the release of encapsulated materials was recorded during the time of storage.

1. Manufacture

For manufacture of MVL encapsulating cytosine
20 arabinoside with sucrose as an osmotic spacer in the presence of 0.1 N HCl, the lipid combination solution contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, and the triglyceride component was either 1.1 g tricaprin (C10), 1.3 g trilaurin (C12), or 1.7 g
25 triolein (molar ratio of DOPC:DPPG:cholesterol:triglyceride was 0.34:0.07:0.52:0.06).

The first aqueous phase solution contained (per ml) 20 mg cytosine arabinoside and 51.3 mg sucrose in 0.1
30 N Hcl. A first emulsion was made by high-speed mixing of

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3 ml of lipid combination solution with 3 ml of aqueous phase solution at 9000 rpm for 8 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by addition of 20 ml of a 20 mM lysine, 4% glucose solution to the mixing vessel and mixing at 5000 rpm for 1.5 min. The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 70 ml of a 40 mM lysine, 3.2% glucose solution, placing the flask in 37 C gyrorotary water bath and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes to form the MVL particles in suspension.

The suspensions were diluted 1:4 by volume with normal saline, and the particles were harvested by centrifugation at 800 X g for 10 minutes at room temperature. The supernatant solution was removed by aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation. The final washed product was resuspended at 25% packed-particle volume per total volume and stored at 2-8, 22, and 37°C for 1 or 6 days (data not shown). The suspension and the supernatant fraction obtained by centrifugation at 25,000 X G 2 minutes were analyzed for content of cytosine arabinoside by 1:1 volume dilution with 50% isopropyl alcohol, vortexing, incubation at 37°C for 10 min, and centrifugation. Then 0.06 or 0.2 ml of the sample was added to 1.0 ml of 0.1 N HCl, and cytosine arabinoside concentration was measured by absorbance determined at 280 nm.

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TABLE 3

Triglyceride Component	Storage Temp °C	Total AraC Encapsulated mg/ml	Supernatant AraC @24h mg/ml	Ratio of Supernatant to total (Sup/Total)
5 Triolein, mp 5°C	2-8	6.0	0.4	0.06
	22	5.6	0.3	0.05
	37	5.2	0.9	0.17
Trilaurin, mp 46°C	2-8	3.0	2.5	0.82
	22	3.1	2.0	0.65
	37	3.0	2.3	0.78
Tricaprin, mp 31°C	2-8	5.6	5.0	0.90
	22	5.4	4.8	0.90
	37	6.7	0.6	0.09

10 As shown by the data in Table 3, the product yield of the MVL formulated using each of the neutral lipids was at least 3.0 mg/ml, an acceptable yield. Inspection of the particles with white light microscopy showed that the particles were spherical and multivesiculated in
 15 appearance immediately post-manufacture. However, within 24 hours of storage at temperatures below the melting point of the triglyceride, the particles changed in appearance. Associated with the change in appearance was significant loss of the encapsulated AraC to the storage
 20 solution (supernatant) as can be seen by the data in column 3 of Table 3. Noteworthy, the formulation manufactured with tricaprin (mp. 31°C) did not change in appearance, or lose significant AraC when stored at 37°C; whereas the formulation with trilaurin (mp 46°C) did

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undergo a significant morphological change and loss of encapsulated active agent. Thus in formulating MVL for *in vivo* controlled and sustained delivery of the encapsulated active agent, the neutral lipid selected
5 should usually have a melting point below or near the intended storage temperature. A preferred formulation would maintain a supernatant/total ratio of less than 0.10 during storage.

Example 16

10 This example illustrates that certain aqueous phase solutions are interactive with the lipid layer membrane of MVLs and prevent freezing of the liposomes at storage temperatures below the melting point of the neutral lipid. In such cases, the morphological
15 transition of the particles and loss of encapsulated materials associated with storage below the melting point of the neutral lipid may occur very slowly, or disappear. Instead the release of encapsulated materials is associated with activation by storage temperature
20 increase.

1. Manufacture

For manufacture of MVL encapsulating rhu IGF with ¹⁴C-sucrose as the osmotic spacer and ammonium citrate as the buffer, the lipid combination solution contained (per
25 liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, and the triglyceride component was 1.3 g tricaprln (mp 31°C, C10) (with the molar ratio of DOPC:DPPG:cholesterol:triglyceride was 0.34:0.07:0.52:0.06).

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The first aqueous phase solution contained (per ml) 16 mg rhu IGF-1 (Chiron, Foster City, CA), 7% ¹⁴C-sucrose, and 20 mM ammonium citrate, pH 5. A first emulsion was made by high-speed mixing of 5 ml of lipid combination solution with 5 ml of aqueous phase solution at 9000 rpm for 9 minutes at 25-27°C. The emulsion was sheared into microdroplets (spherules) by addition of 30 ml of a 40 mM lysine, 4% glucose solution to the mixing vessel and mixing at 6000 rpm for 1 min. The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 70 ml of a 40 mM lysine, 3.2% glucose solution, placing the flask in a gyrorotary water bath at 37°C, and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes to obtain the MVL particles.

The suspensions were diluted 1:4 with normal saline and the particles were harvested by centrifugation at 800 X g for 10 minutes at room temperature. The supernatant solution was removed by aspiration, and the particles were washed twice by resuspension in fresh, normal saline solution and centrifugation.

2. In vitro release rates

The final washed product was resuspended at 25% packed-particle volume per total volume and stored at temperatures of 2-8, 25, 32, and 37°C for 24 or 48 hours. The pellet and supernatant fraction from the centrifugation at 25,000 X G for 2 minutes was assayed for content of IGF-1 (pellet fraction only) and ¹⁴C-sucrose.

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TABLE 4

Temperature, °C	Retained @ 24 hours		Retained @ 48 hours	
	¹⁴ C-Sucrose	IGF-1	¹⁴ C-Sucrose	IGF-1
2-8	1.00	1.00	0.98	0.98
5 25	0.95	0.97	0.91	0.94
32	0.90	0.96	0.74	0.86
37	0.83	0.91	0.56	0.75

The results of these studies summarized in Table 4 show that the contents of the first aqueous phase solution can prevent or slow the freezing effect which was shown in Example 16 to be associated with storage of MVLs containing trilaurin as the neutral lipid at a temperature below its melting point of 46°C. The lipids used and method of manufacture were identical in both formulations; however, the encapsulated aqueous phase components were different. This suggests that the composition of the first aqueous phase may modulate either the freezing point of the triglyceride or the melting point effect, for instance by preventing the neutral lipid from transitioning to an unstable structure or markedly slowing the freezing.

The release in plasma of the tricaprln- and trilaurin-containing MVL formulations (shown in Example 16) was markedly slower than from MVL manufactured with tricaprlylin, as shown in Table 4 and in later examples. It should be noted that all the plasma release assays conducted at 37°C were initiated within 24 hours of manufacture. The storage at 2-8°C for longer than a few days of the trilaurin, but not the tricaprln-containing formulations, resulted in morphological change in the

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appearance of the particles and release of encapsulated materials consistent with the "melting point effect".

Example 17

Other neutral lipids

5 A study series was performed wherein the neutral lipids were decane, dodecane, squalene, and alpha-tocopherol to determine the scope of neutral lipids that can be used to obtain multivesicular liposomal formulations.

10 1. Manufacture

 Multivesicular liposomes encapsulating a combination of glycine, sucrose, and Tris-EDTA were made wherein the lipid combination solution contained (per liter) 10.4 g DOPC, 2.1 g DPPG, 7.7 g cholesterol, and
15 the triolein component (6 mol % of lipid) was replaced with either decane, dodecane, squalene, or alpha-tocopherol, (molar ratio of DOPC:DPPG:cholesterol:neutral lipid was 0.34:0.07:0.52:0.06).

 The first aqueous phase solution contained 200 mM
20 glycine, 50 mM Sucrose, 1.8 mM Tris base, and 0.5 mM EDTA, pH 7.44, with an osmolarity of 268 mOsmol. A first emulsion was made by high-speed mixing of 3 ml of lipid combination solution with 3 ml of aqueous phase solution at 9000 rpm for 9 minutes at 25-27°C. The emulsion was
25 sheared into microdroplets (spherules) by the addition of 20 ml of a 20 mM lysine, 4% glucose solution to the mixing vessel and mixing at 4000 rpm for 1.0 min.

 Examination of the spherule suspensions under the microscope indicated that the spherules prepared with
30 each of these neutral lipids were normal in internal

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appearance as compared to controls prepared with triolein, tripalmitolein, or trimyristolein as the neutral lipid component.

The chloroform was removed from the microscopic droplets or spherules by transferring the suspension to a flask containing 30 ml of a 40 mM lysine, 3.2% glucose solution, placing the flask in 37°C gyrorotary water bath, and flushing the surface of the suspension with nitrogen gas at a flow rate of 70 cfh for 20 minutes to obtain the MVL particles.

Only MVL prepared with squalene as the neutral lipid survived the solvent removal step or subsequent washing of the particles and gave rise to normal-looking MVL particles after the wash step. During the solvent removal step, decane, dodecane and alpha-tocopherol spherules began to shrink, with lobes of light-refractile material emanating from their surface. Abruptly, the particles collapsed into a crenellated structure. In some cases a pellet, albeit small as compared to controls, was recovered from the wash step, and the particles did not have the appearance of multivesicular liposome compositions.

Other Embodiments

The foregoing description of the invention is exemplary for purposes of illustration and explanation. It should be understood that various modifications can be made without departing from the spirit and scope of the invention. Accordingly, the following claims are intended to be interpreted to embrace all such modifications.

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CLAIMS:

1. A method for modifying the rate of release of a biologically active compound encapsulated in a multivesicular liposome having a neutral lipid component
5 comprising:

(1) forming an emulsion from a) a lipid component comprising an organic solvent, an amphipathic lipid, and a neutral lipid component comprising a molar ratio of from 1:0 to 0:1 of a slow release rate neutral lipid to a
10 fast release rate neutral lipid, and b) an immiscible first aqueous component wherein at least one biologically active compound is incorporated into either the lipid component or the first aqueous component, or both;

(2) mixing the emulsion with a second aqueous
15 component to form solvent spherules, and

(3) removing the organic solvent from the solvent spherules to form multivesicular liposomes encapsulating the biologically active compound;

wherein the molar ratio of the slow release
20 neutral lipid to the fast release neutral lipid is decreased to increase the rate of release of the biologically active compound.

2. The method of claim 1, wherein the molar
25 ratio of the neutral lipid component to all the lipids in the liposome is in the range from about 0.01 to about 0.21.

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3. The method of claim 1, wherein the slow release neutral lipid is selected from the group consisting of triglycerides having a monounsaturated fatty acid ester containing from about 14 to 18 carbons
5 in the acyl chain, those having a saturated fatty acid ester containing from about 10 to 12 carbons in the acyl chain, and mixtures thereof.

4. The method of claim 1, wherein the slow release neutral lipid is selected from the group
10 consisting of triglycerides having a monounsaturated fatty acid ester containing from about 6 to 8 carbons in the acyl chain.

5. The method of claim 1, wherein the molar ratio of the slow release neutral lipid to the fast
15 release neutral lipid is in the range from about 1:1 to 1:100.

6. The method of claim 1, wherein the molar ratio of the slow release neutral lipid to the fast release neutral lipid is in the range from about 1:4 to
20 1:27.

7. The method of claim 1, wherein the slow release neutral lipid is selected from the group consisting of triolein, tripalmitolein, trimyrystolein, trilaurin, tricaprin, and mixtures thereof, and the fast
25 release neutral lipid is selected from the group consisting of tricaprylin, tricaproin, and mixtures thereof.

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8. The method of claim 1, wherein the slow release neutral lipid is tripalmitolein.

9. The method of claim 1, wherein the slow release neutral lipid is triolein.

5 10. The method of claim 1, wherein the slow release neutral lipid is tricaproin.

11. The method of claim 1, wherein the fast release neutral lipid is selected from the group
10 consisting of triglycerides having a saturated fatty acid ester containing from 6 to 8 carbons in the acyl chain.

12. The method of claim 1, wherein the fast release neutral lipid is selected from the group consisting of tricaprylin, tricaproin, and mixtures
15 thereof.

13. The method of claim 9, wherein the fast release neutral lipid is tricaprylin or a mixture of tricaprylin and tricaproin.

14. The method of claim 1, wherein the slow
20 release neutral lipid is selected from the group consisting of C8,C10 mixed acyl propylene glycol diesters and cholesterol esters and the fast release neutral lipid is tricaprylin or tricaproin.

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15. A method for modifying the rate of release of a biologically active compound encapsulated in a multivesicular liposome, said method comprising utilizing a blend of triolein or tripalmitolein and a fast release neutral lipid as the neutral lipid component in a multivesicular formulation in which an active compound is encapsulated;

wherein the rate of release of the biologically active compound increases in proportion with the molar ratio of the fast release neutral lipid to the triolein or tripalmitolein in the neutral lipid component.

16. The method of claim 15, wherein the rate of release is *in vivo*, and the neutral lipid component has a melting point about or below an *in vivo* temperature.

17. The method of claim 15, wherein the release is at storage temperature, and the melting point of the neutral lipid component is about or above the storage temperature.

18. The method of claim 15, wherein the fast release neutral lipid is selected from the group consisting of tricaprylin, tricaproin, and mixtures thereof.

19. The method of claim 15, wherein the fast release neutral lipid is tricaprylin, and the molar ratio of triolein or tripalmitolein to tricaprylin is in the range from about 1:1 to 1:100.

20. The method of claim 15, wherein the molar

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ratio of the triolein or tripalmitolein to the fast release neutral lipid is in the range from about 1:1 to 1:27.

21. The method of claim 15, wherein the release is *in vivo*, and the fast release neutral lipid is tricaprylin.

22. A method for selecting a multivesicular liposome formulation with a desired release rate of an encapsulated biologically active compound at a given temperature, said method comprising:

(a) preparing a family of multivesicular liposomal formulations wherein each member of the family is made by

(1) forming an emulsion from (i) a lipid component comprising an organic solvent, an amphipathic lipid, and a neutral lipid component comprising a molar ratio of from 1:0 to 0:1 of a slow release rate neutral lipid to a fast release rate neutral lipid, and (ii) an immiscible first aqueous component, wherein at least one biologically active compound is incorporated into either the lipid component or the first aqueous component, or both;

(2) mixing the emulsion with a second aqueous component to form solvent spherules, and

(3) removing the organic solvent from the solvent spherules to form multivesicular liposomes encapsulating the biologically active compound;

wherein for each member of the family the neutral lipid component has a different molar ratio of the slow release rate neutral lipid to the fast release rate neutral lipid;

(b) incubating each member of the family at a given temperature to obtain a family of release rate profiles; and

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(c) selecting the family member with the neutral lipid component yielding the desired release rate profile.

23. The method of claim 22, wherein the
5 amphipathic lipid is selected from the group consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (DEPC), and the slow release neutral lipid is triolein or tripalmitolein.

10 24. The method of claim 22, wherein the fast release neutral lipid is tricaprylin.

25. The method of claim 22, wherein the molar ratio is selected in the range from about 1:1 to 1:54.

15 26. The method of claim 22, wherein the molar ratio of the neutral lipid component to all the lipids in the lipid component is in the range from about 0.01 to about 0.21.

20 27. The method of claim 22, wherein the slow release neutral lipid is selected from the group consisting of triolein, tripalmitolein, trimyristolein, trilaurin, tricaprin and mixtures thereof, and the fast release neutral lipid is selected from the group consisting of tricaprylin, tricaproin, and mixtures thereof.

25 28. The method of claim 27, wherein the molar ratio of the slow release neutral lipid to the fast release neutral lipid is in the range from about 1:1 to 1:54.

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29. The method of claim 22, wherein the release rate is *in vivo* and the slow release neutral lipid is triolein.

30. The method of claim 29, wherein the fast
5 release neutral lipid is tricaprylin.

31. The method of claim 22, wherein the amphipathic lipid is selected from the group consisting of

1,2-dioleoyl-*sn*-glycero-3-phosphocholine,
10 1,2-dilauroyl-*sn*-glycero-3-phosphocholine,
1,2-dimyristoyl-*sn*-glycero-3-phosphocholine,
1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine,
1,2-distearoyl-*sn*-glycero-3-phosphocholine,
1,2-diarachidoyl-*sn*-glycero-3-phosphocholine,
15 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine,
1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine,
1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine,
1,2-dierucoyl-*sn*-glycero-3-phosphocholine,
1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, and
20 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol.

32. A multivesicular liposome comprising:
a neutral lipid component having a molar
ratio of a slow release neutral lipid to a fast neutral
lipid in the range from about 1:1 to 1:100;
25 an amphipathic lipid; and
a biologically active compound encapsulated
in water in the liposome.

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33. The liposome of claim 32, wherein the melting point of the neutral lipid component is about or below 37°C.

34. The liposome of claim 32, wherein the slow
5 release neutral lipid is selected from the group consisting of triglycerides having a monounsaturated fatty acid ester with from about 14 to 18 carbons in the acyl chain, those having a saturated fatty acid ester with from about 10 to 12 carbons in the acyl chain, and
10 mixtures thereof.

35. The liposome of claim 32, wherein the slow release neutral lipid is selected from the group consisting of triolein, tripalmitolein, trimyristolein, trilaurin, tricaprln and mixtures thereof, and the fast
15 release neutral lipid is selected from the group consisting of tricaprylin, tricaproin, and mixtures thereof.

36. The liposome of claim 32, wherein the fast release neutral lipid is selected from the group
20 consisting of triglycerides having a saturated fatty acid ester with from about 6 to 8 carbons in the acyl chain.

37. The liposome of claim 32, wherein the fast release neutral lipid is selected from the group consisting of tricaprylin and tricaproin, and mixtures
25 thereof.

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38. The liposome of claim 32, wherein the molar ratio of the neutral lipid component to all the lipids in the liposome is in the range from about 0.01 to about 0.21.

5 39. The liposome of claim 32, wherein the molar ratio of the slow release neutral lipid to the fast release neutral lipid is in the range from about 1:1 to 1:27.

40. The liposome of claim 32, wherein the molar
10 ratio of the slow release neutral lipid to the fast release neutral lipid is in the range from about 1:1 to 1:18.

41. A method for administering a biologically active compound at a desired release rate comprising
15 administering the liposome of claim 32 to a subject in need of the biologically active compound encapsulated therein.

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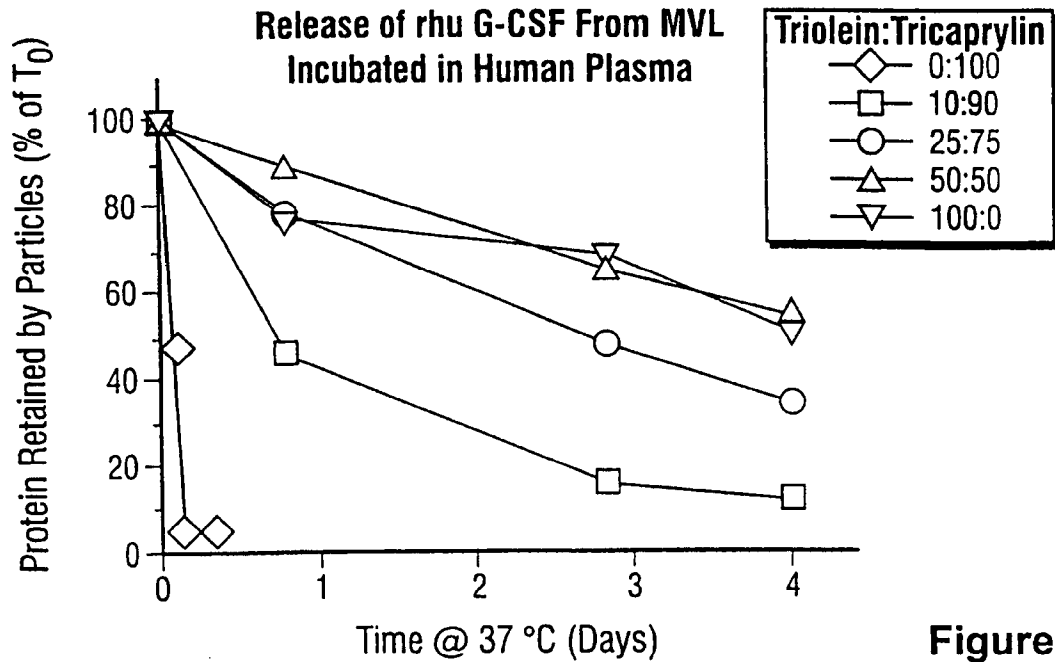


Figure 1

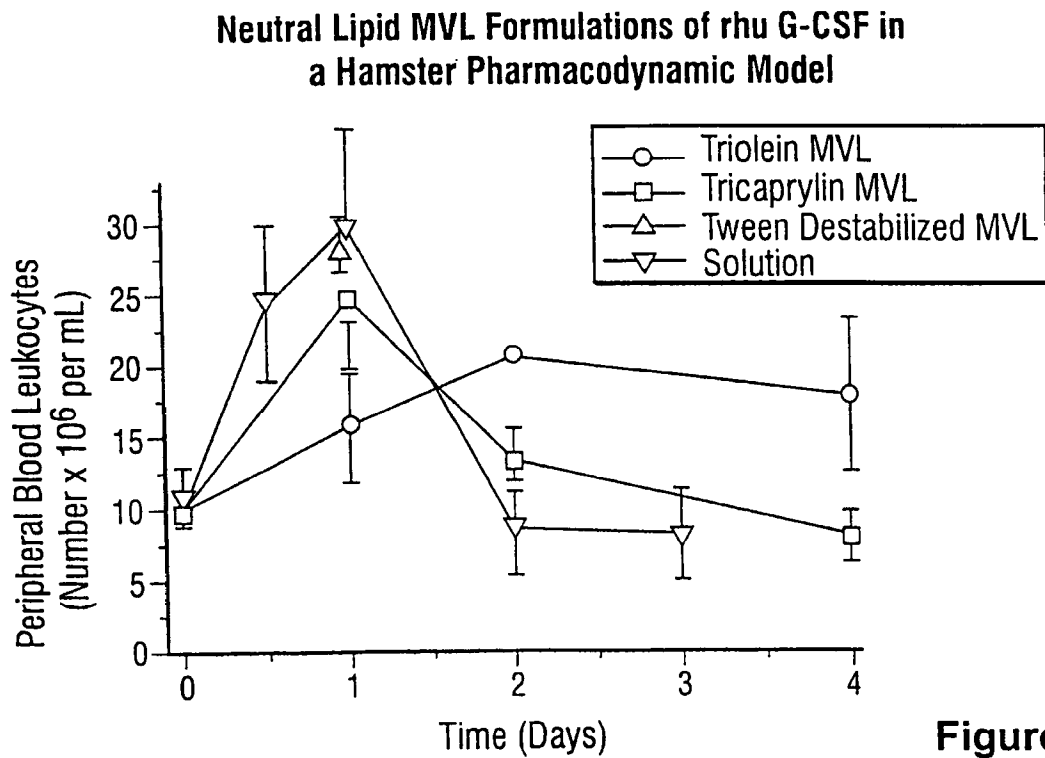


Figure 2

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Release of rhu GM-CSF from MVL
Incubated in Human Plasma

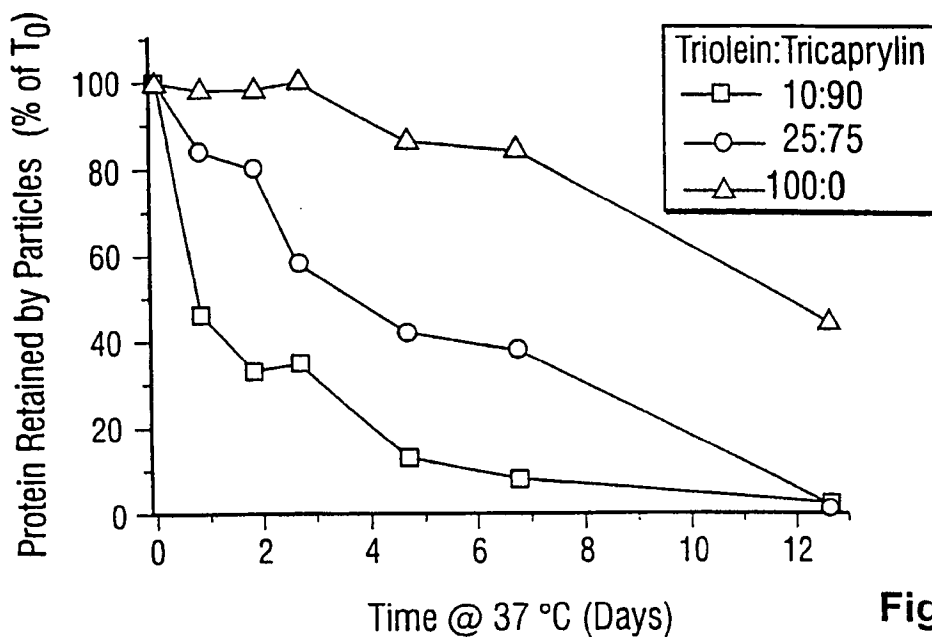


Figure 3

Neutral Lipid MVL Formulations of rhu GM-CSF in
a Marine Pharmacokinetic Model

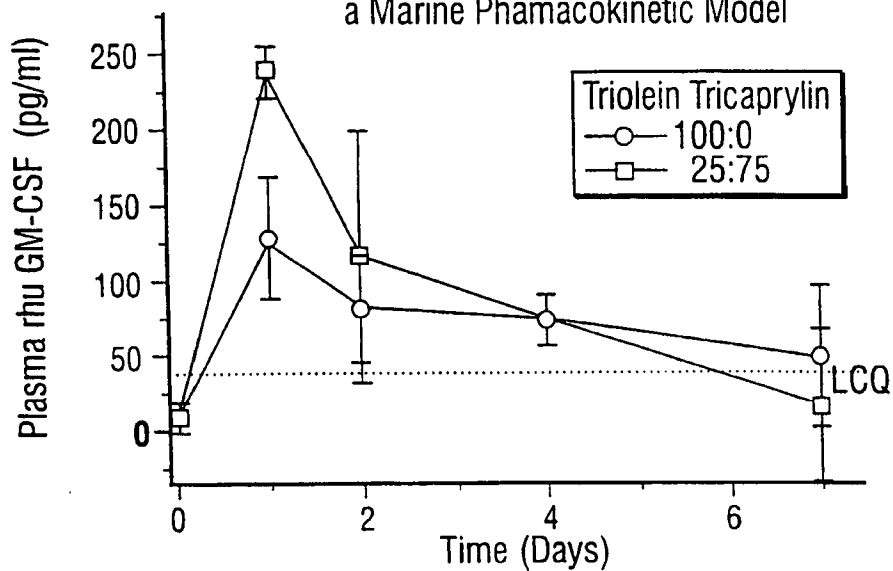


Figure 4

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Release of rhu IGF-1 in Human Plasma
From MVL Manufactured with Different Neutral Lipids

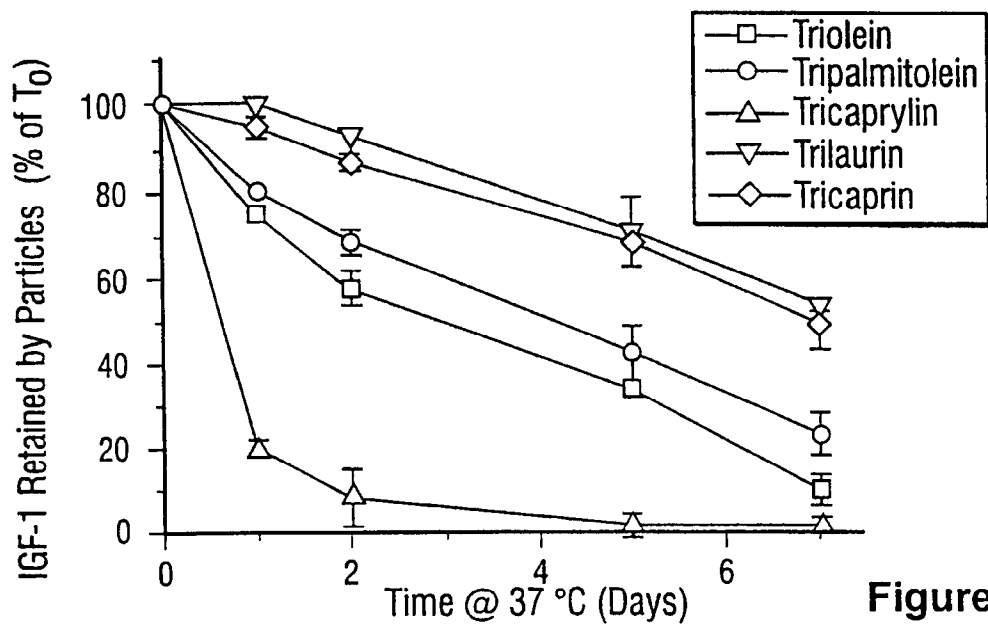


Figure 5

MVL Formulations of rhu IGF-1 in
a Rat Pharmacokinetic Model

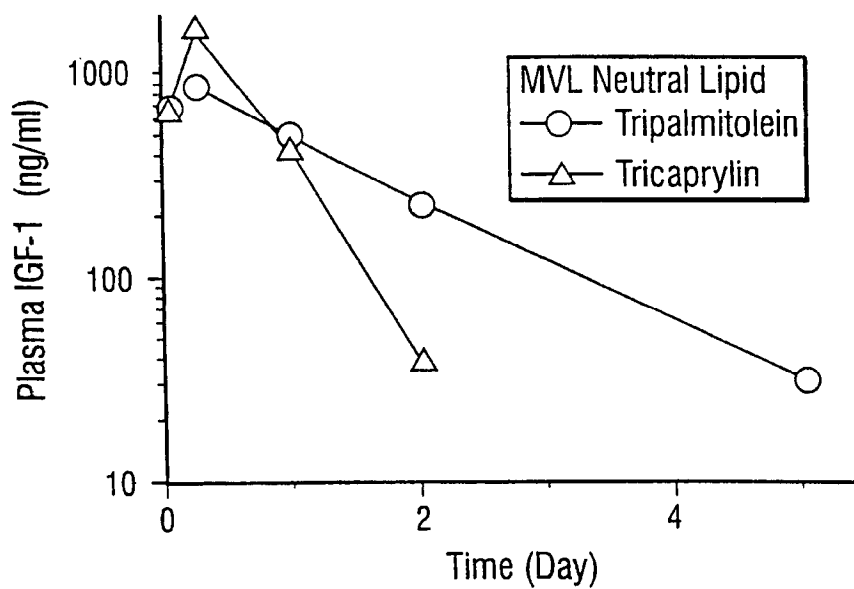


Figure 6

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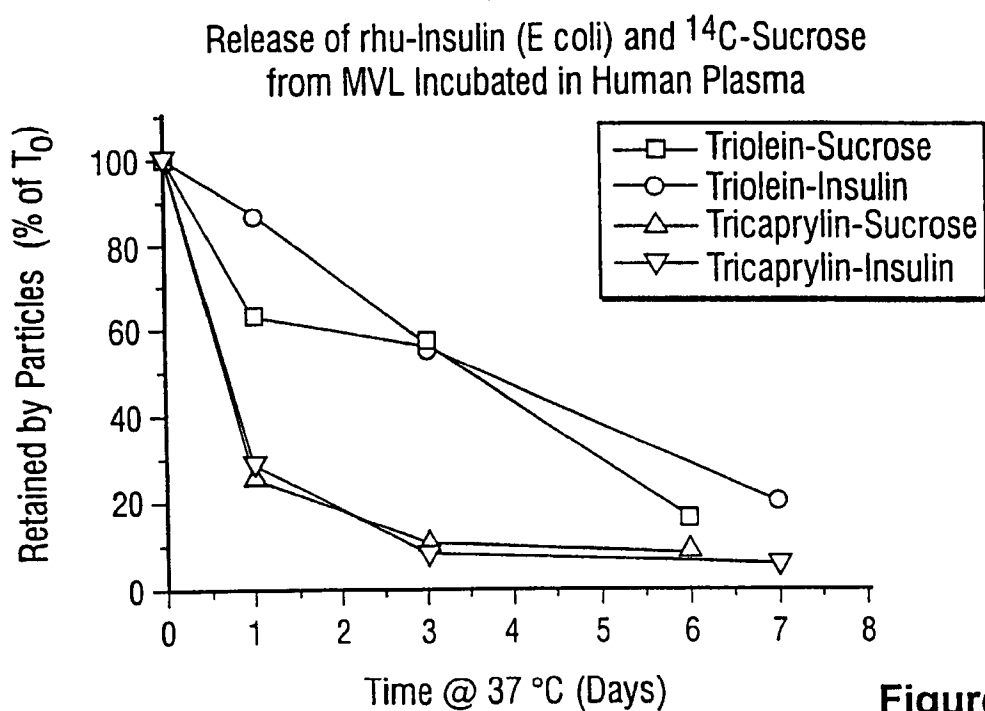


Figure 7

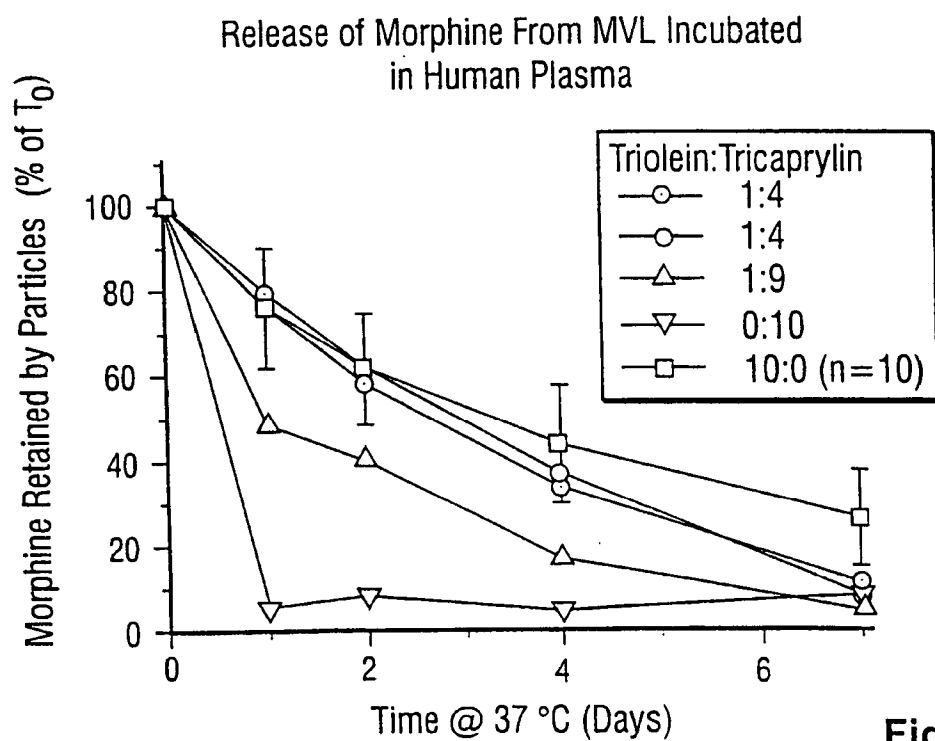


Figure 8

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Morphine Levels in Dog CSF Following Epidural Injection of
Morphine Sulfate in Different Neutral Lipid MVL Formulations

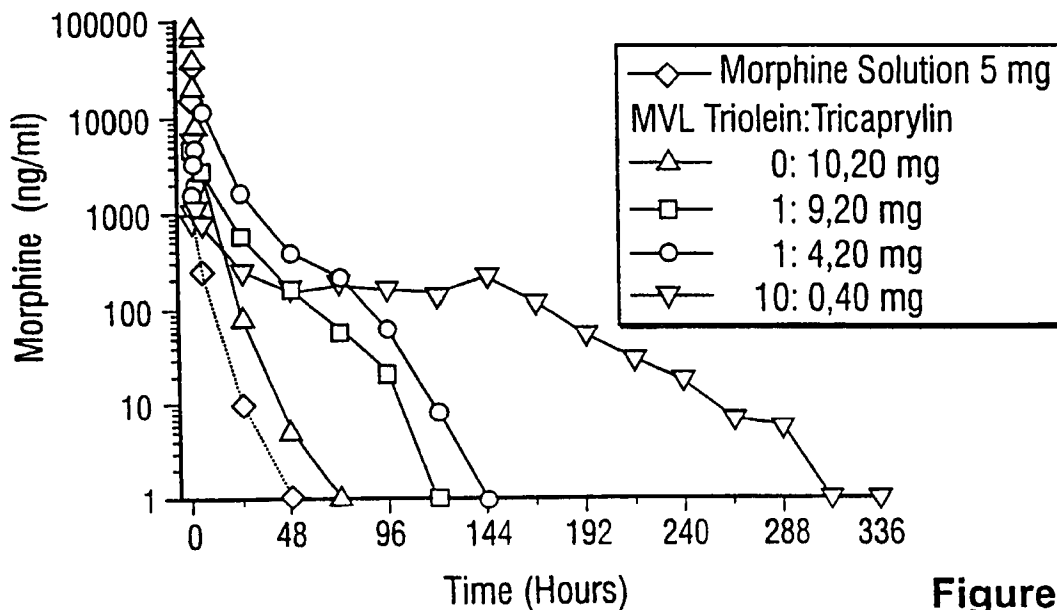


Figure 9

The Release Rate of Morphine MVL Formulations
Stored at Different Temperatures in Normal Saline Solutions

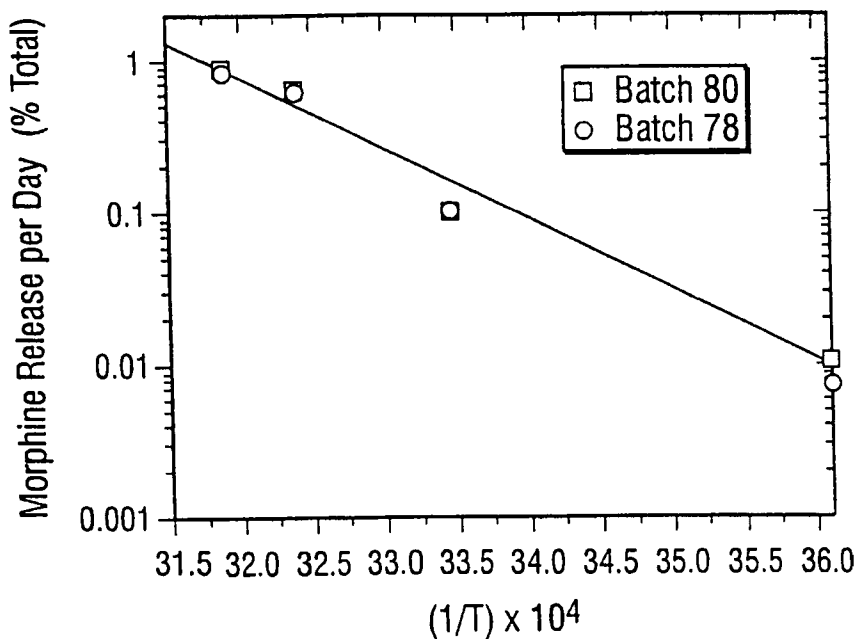


Figure 10

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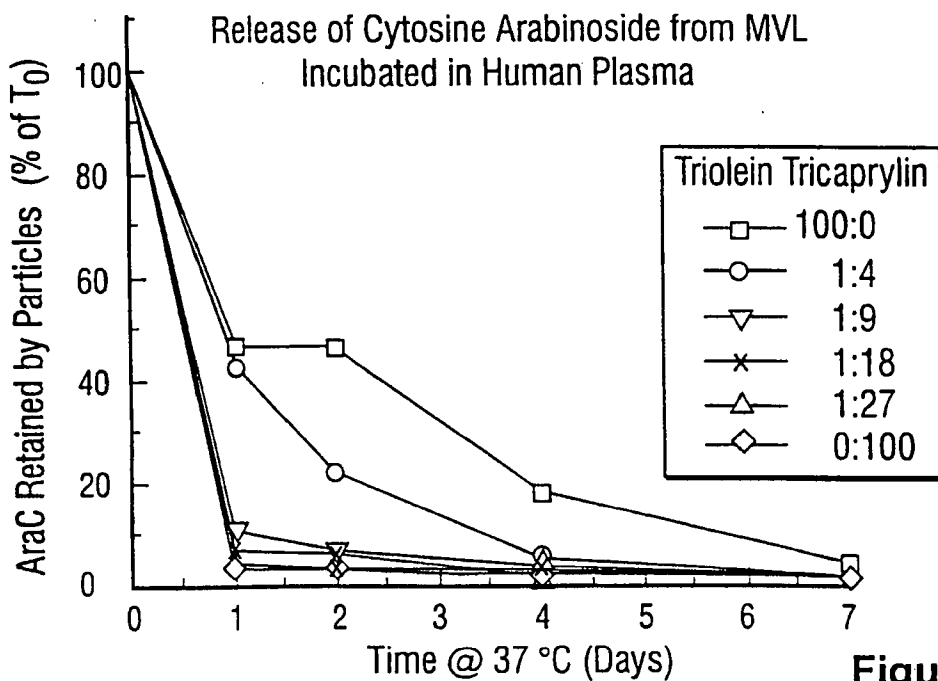


Figure 11

The Influence of Phospholipid Chain-Length Composition on the Neutral Lipids
Effect on MVL Release of Cytosine Arabinoside (AraC) in Human Plasma:

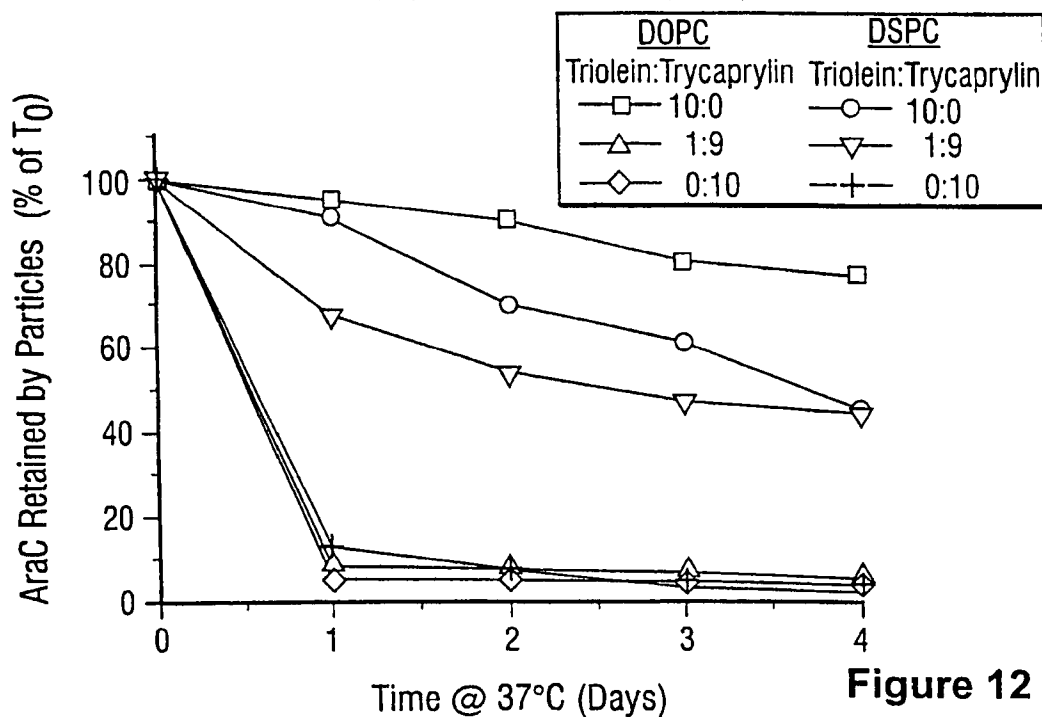
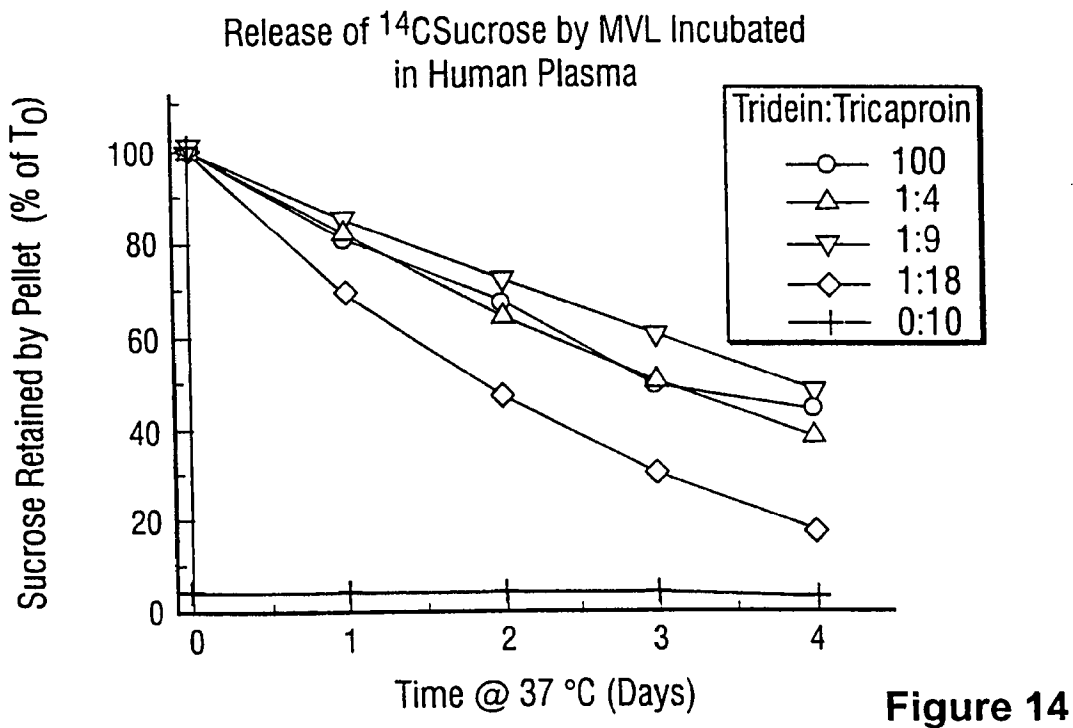
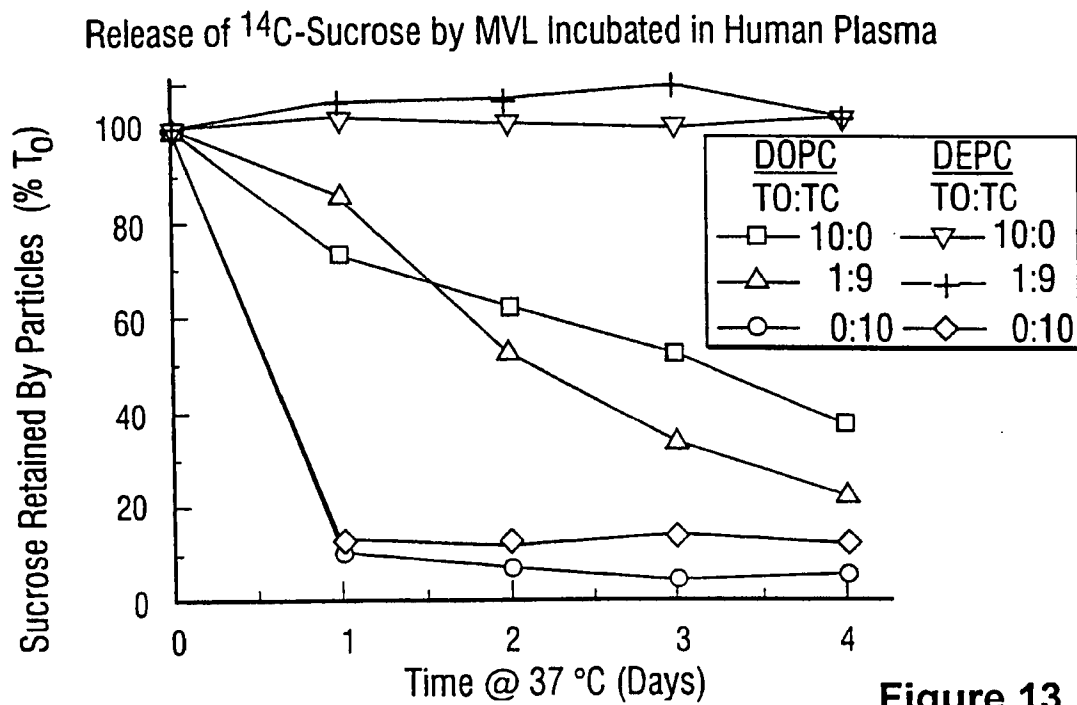


Figure 12

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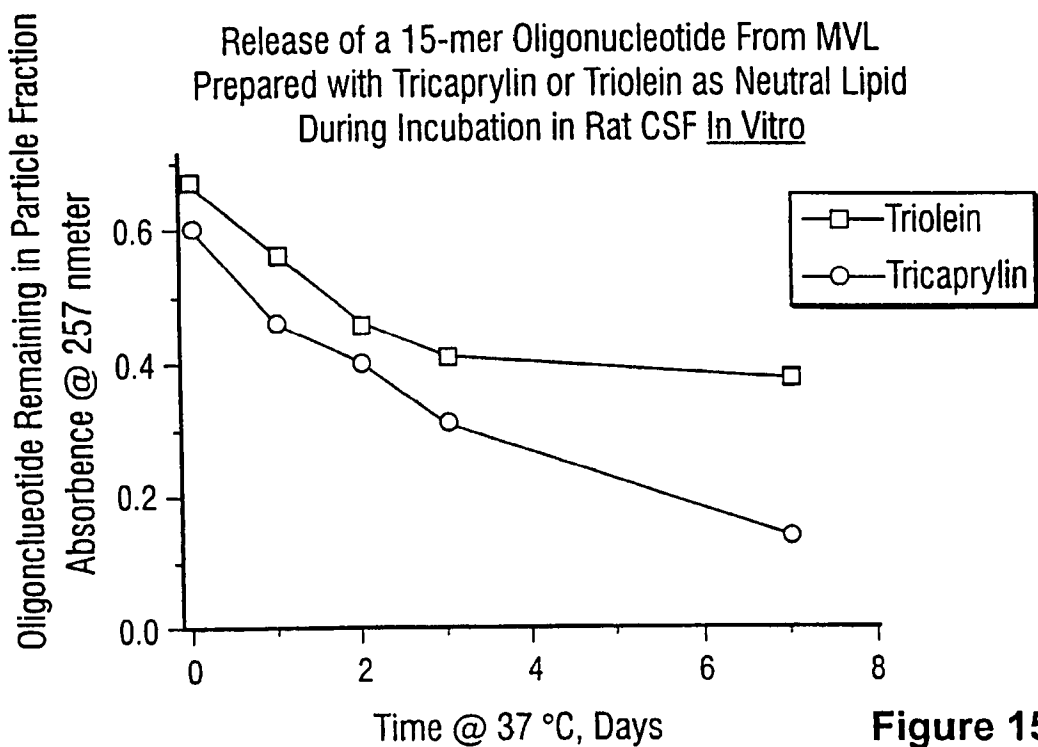


Figure 15

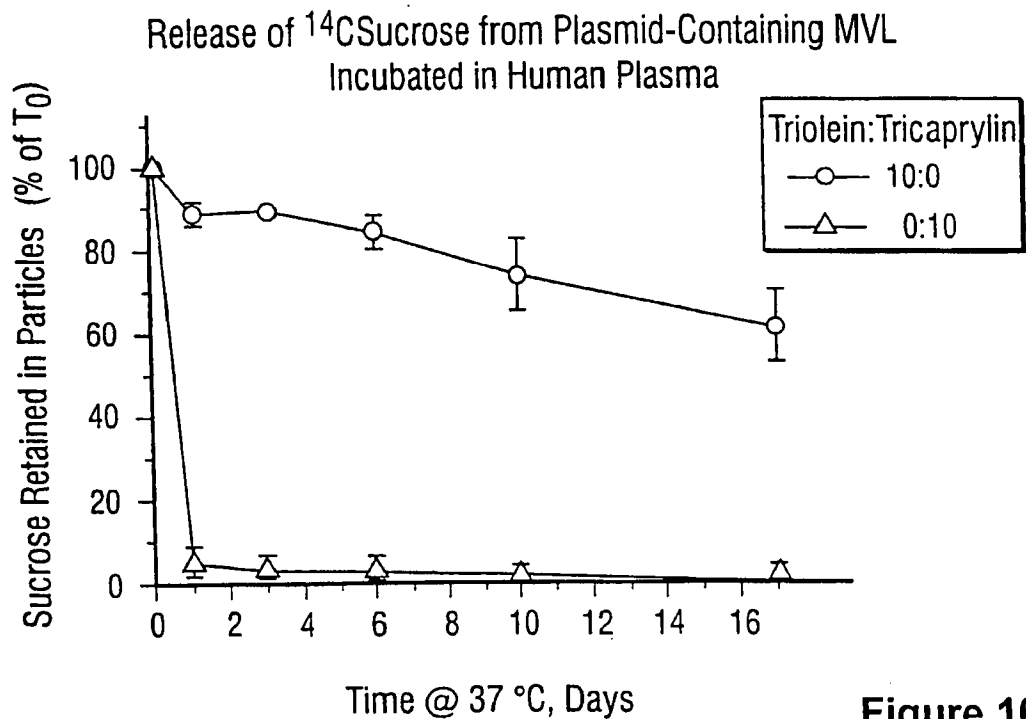


Figure 16

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Release of ^{14}C -Sucrose From MVL Incubated
in Human Plasma

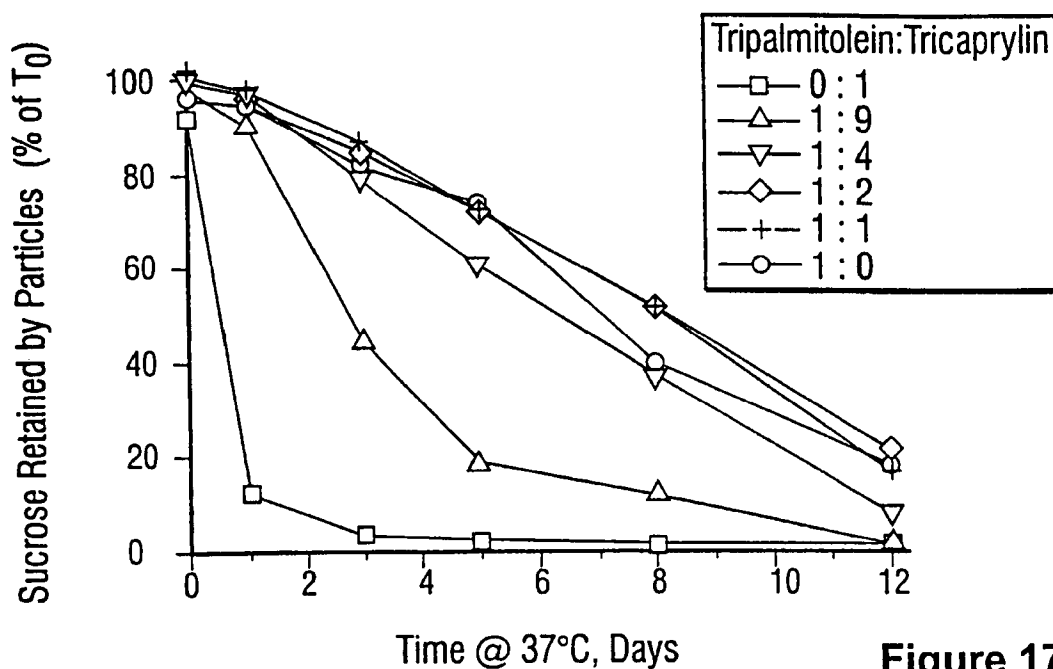


Figure 17

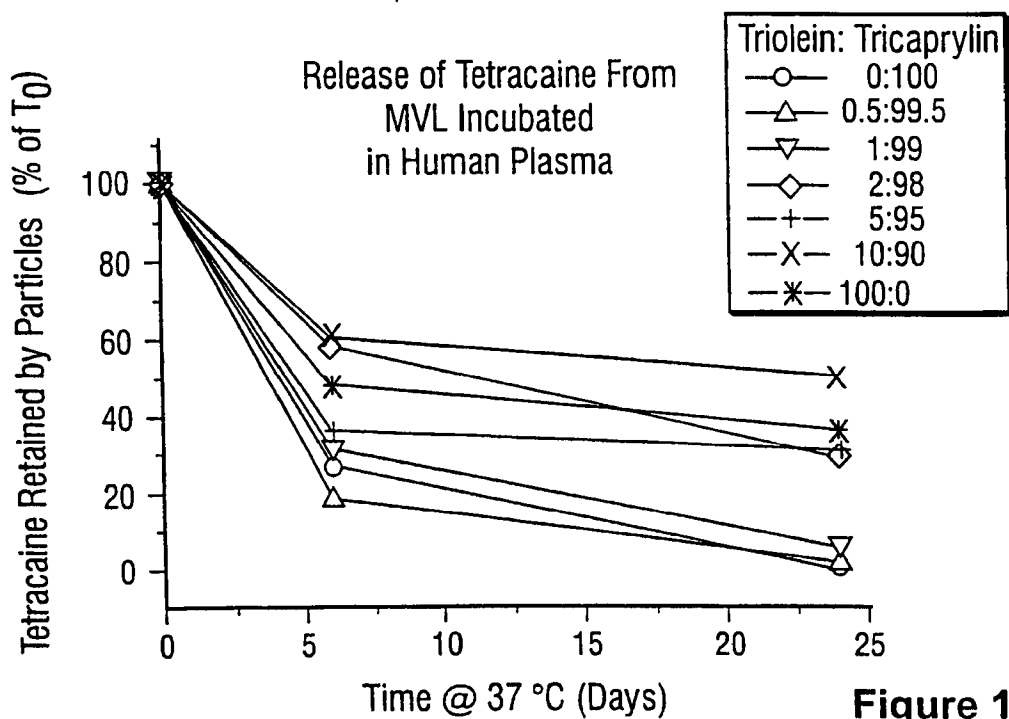
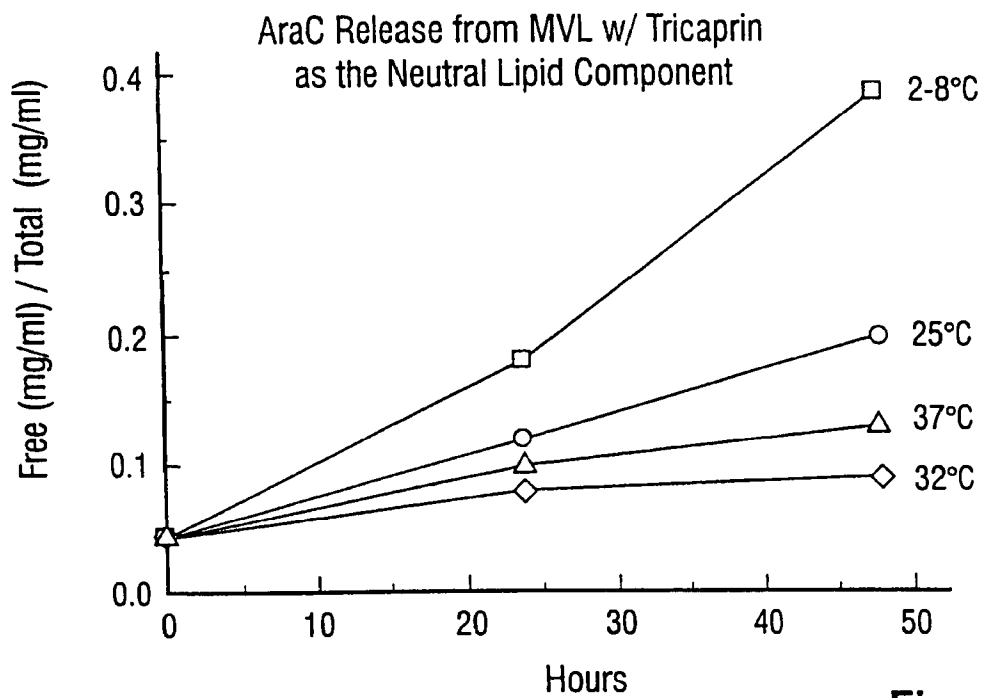
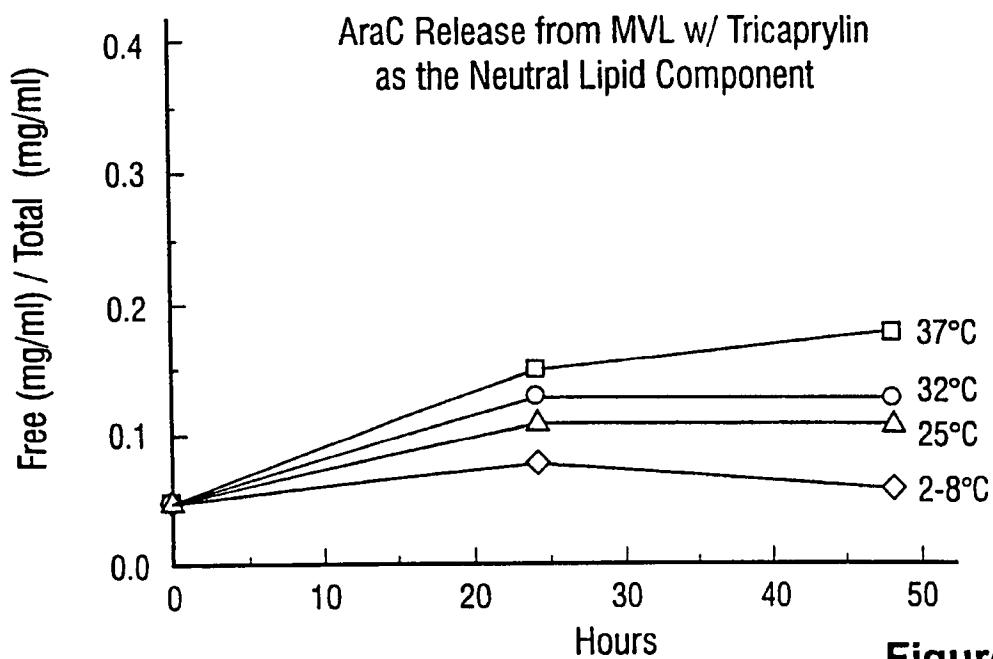


Figure 18

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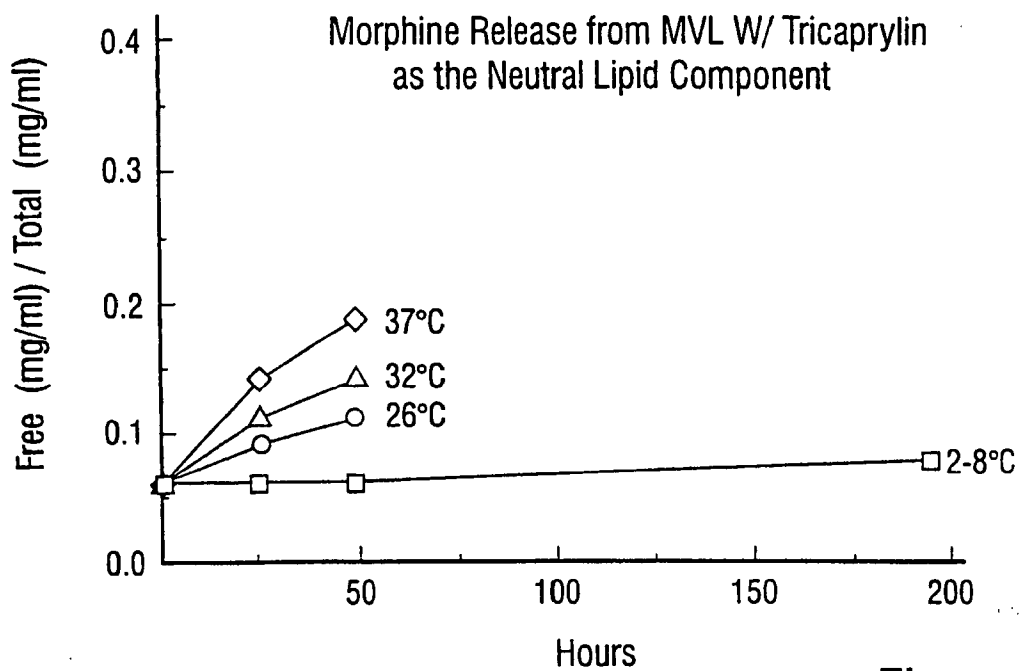


Figure 19C

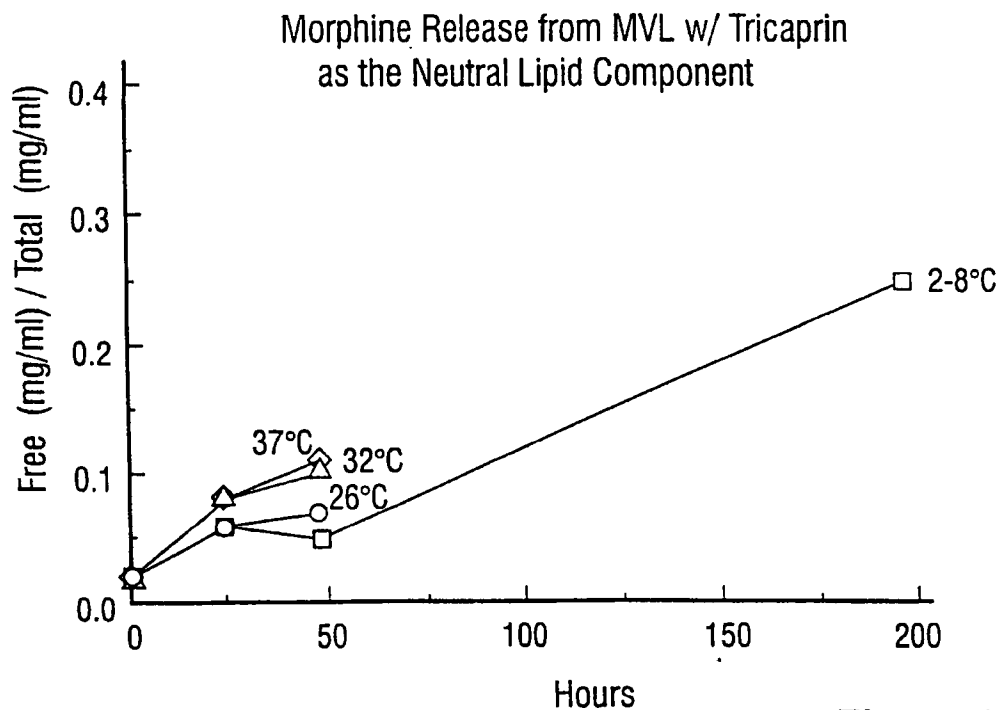
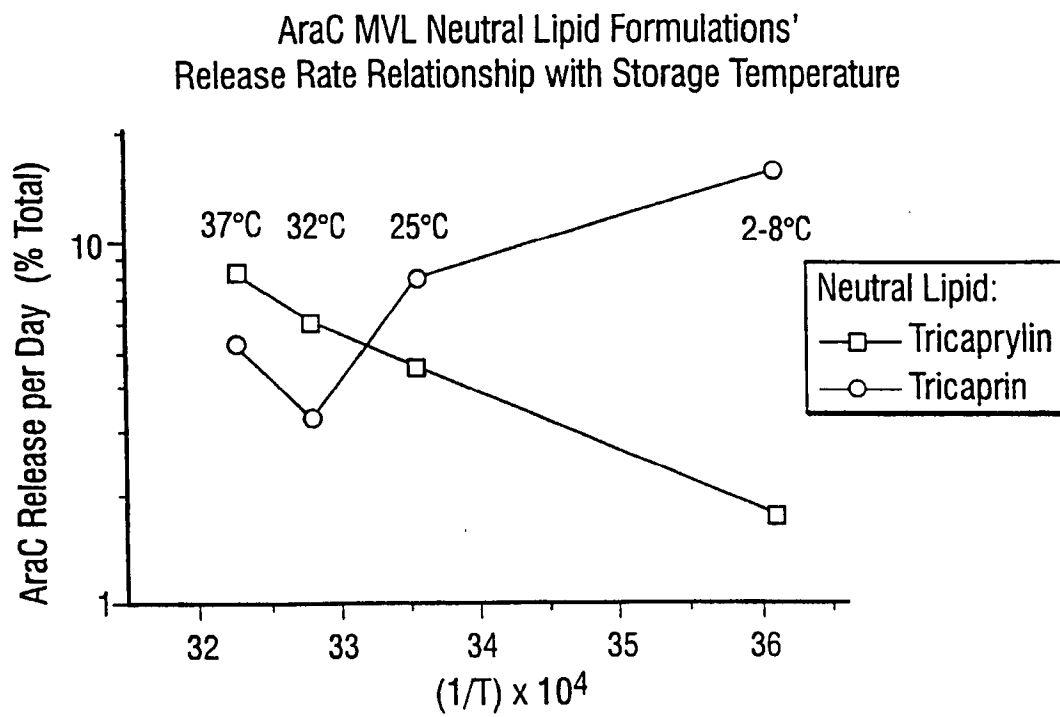


Figure 19D

12/12

**Figure 20**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01636

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 9/127, 9/133

US CL : 424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450; 264/4.1, 4.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS; Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,422,120 A (KIM) 06 June 1995, Examples, especially 5 and 6.	1--7, 9-21, 32-35 & 38-41 ----- 8, 22-31 & 36-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

29 MARCH 1998

Date of mailing of the international search report

28 MAY 1998

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/SE93/00257 (22) International Filing Date: 26 March 1993 (26.03.93) (30) Priority data: 9200952-1 27 March 1992 (27.03.92) SE (71) Applicant (for all designated States except US): KABI PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): NYQVIST, Håkan [SE/SE]; Eklidsvägen 20, S-146 00 Tullinge (SE). RAG-NARSSON, Gert [SE/SE]; Björkvägen 5, S-197 00 Bro (SE). TINGVALL, Per [SE/SE]; Skvadronsbacken 35, S-172 47 Sundbyberg (SE).		(74) Agents: FORSLUND, Niklas et al.; Kabi Pharmacia AB, S-112 87 Stockholm (SE). (81) Designated States: FI, JP, NO, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: A PHARMACEUTICAL CARRIER SYSTEM CONTAINING DEFINED LIPIDS (57) Abstract The present invention relates to a lipid carrier system for a local anaesthetic comprising a defined lipid system of at least two lipid components wherein at least one of the lipid components is amphiphatic and polar and one is nonpolar. The system may further contain a hydrophilic solvent and additives or matrices for adapting it for administration to mucous membranes and for transdermal administration.		

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A PHARMACEUTICAL CARRIER SYSTEM CONTAINING DEFINED LIPIDS

Field of invention

The present invention relates to a lipid carrier system for a local anaesthetic comprising a defined lipid system which includes at least two lipid components, wherein at least one of the lipid components is polar and amphiphatic and one is nonpolar. The system may further include a hydrophilic solvent and additives or matrices for adapting the system for administration to mucous membranes and for transdermal administration.

Description of the invention

According to the invention the lipid carrier system, referred to as Biosome Forming Matrix (BFM), is characterized by a defined system of at least two defined lipid components chosen from classes of different polarity, of which at least one of the lipid components is bilayer forming. By this is meant that discrete lipid particles, referred to as Biosomes, are formed spontaneously when the system interacts with excess aqueous media. This lipid system is also described in the International Patent Application WO 92/05771. By a defined lipid component is meant a lipid whose chemical composition is known and controlled. This will be explained in more detail below.

As before mentioned, at least one of the lipid components of the system is polar and amphiphatic and one is nonpolar. The amphiphatic and polar component is preferably phosphatidylcholine and the nonpolar is preferably chosen from the classes of mono-, di- and triglycerides or a mixture thereof.

The amount of the polar lipid class components will preferably be present in an amount that corresponds to 0.5-90 % (w/w) of the lipid system, preferably corresponding to the range of 5-50 % (w/w).

The property 'bilayer forming' is a well-known physical parameter and can be established readily with suitable physicochemical methods (e.g. surface balance method). The establishment of the formed discrete lipid particles can be done by physical and/or chemical methods, such as microscopy using polarized light, or diffraction methods.

The variation in the lipid composition provides the control mechanism by means of which Biosomes are formed and thereby also the rate at which Biosomes are formed which, will serve as a controlling factor for either immediate or sustained release of the entrapped or associated bioactive materials.

The lipid system according to the present invention can only be defined in the general terms set forth in Claim 1. The difference between the matrix according to the invention and lipid systems that are already known to art resides in the ability of spontaneously forming Biosomes in contact with excess aqueous media. Thus, the inventive lipid system can be obtained by *a)* using well defined lipid components from at least two different lipid classes and by *b)* designing these lipid components into unique lipid matrices, which form Biosomes *in vivo* when interacting with water.

The following definitions are used in this document:

lipids - a general term for natural or synthetic compounds consisting of acyl carriers, such as glycerol, sphingosine, cholesterol, and others or derivatives thereof, to which one or more fatty acids are or can be linked. Similar molecules that contains a substantial hydrocarbon portion may also be included.

The lipids used for the Biosome Forming Matrices (BFMs) can be grouped in different lipid classes, depending on their polarity, namely:

nonpolar lipid classes - these have no polar head groups. Examples of nonpolar constituents are hydrocarbons, or non-swelling amphiphiles, such as mono-, di- and triacylglycerols, cholesterol, fatty alcohols or cholesterol esters.

polar lipid classes - these have polar head groups and possess surface activity, such as phospholipids or glycolipids. Depending on their specific interactions with water, they are subdivided further into the categories of swelling and soluble amphiphiles.

amphiphatic or amphiphilic lipid classes - such as phospholipids and glycolipids, being surface active.

bilayer forming lipid classes - amphiphatic lipids, such as PC (phosphatidylcholine), sphingomyelin, PI (phosphatidylinositol), with a molecular geometry that preferentially leads to bilayer structures in the presence of water.

The lipids used for the BFM consist of a mixture of lipid classes that are characterized by their different polarities. Polar lipids, such as phospholipids or glycolipids, and nonpolar lipids, such as mono-, di- and triglycerides, are the main constituents in the system although sterols, such as cholesterol, fatty acids, fatty alcohols and esters thereof as well as other lipid classes may also be used. This well defined mixture of lipids from different classes as defined above should not be confused with commercial products such as soybean oil, maize oil or soy lecithin and egg lecithin. In order to obtain the well defined lipid classes the commercial material, such as an oil or a lecithin, is fractionated and then the different lipid classes are admixed as explained in more detail in the examples below.

Furthermore, derivatives of lipids may also be used in combination with the above mentioned lipids. One example of this is polyethylene glycol coupled to phosphatidylethanolamine, which has shown to prolong the circulation time of liposomes being injected into the blood stream. Another example of such a derivative is palmitoylcarnitine, which acts as an absorption enhancer for bioactive substances in the gut.

In the preferred way of initiating the formation of the BFM, the bioactive substance is admixed to a selected lipid, followed by admixing of a lipid of a different polarity. This polar/nonpolar alteration may be continued for as many cycles as necessary in the specific case, involving a range of lipids of different polarities.

The preferred way of incorporating a bioactive substance into the BFM is to admix the bioactive substance to amphiphilic lipids so as to create a homogeneous formulation, where the amount of amphiphilic lipids is generally in the total range of 0.5-90 % (w/w). Such an amphiphilic lipid will preferably be capable of spontaneous bilayer formation. Examples thereof are amphiphilic and polar lipid classes, such as phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol or phosphatidylserine or mixtures thereof.

In order to prevent or to delay an immediate interaction of the amphiphile(s) with exogenous water, the system should also contain one or more lipids of the nonpolar lipid class. Examples of such nonpolar lipids are mono-, di- or triglycerols, cholesterol or its esters.

Endogenous water, ethanol or other solvents may be initially present in the system in small quantities (not sufficient for Biosome formation), if the bioactive substance needs such a solvent to be incorporated.

The design of the BFM includes not only the proper selection and/or combination of lipid classes, tailor-made for the solubilization of each bioactive substance, but also the proper selection of the distribution of fatty acids, i.e. the acyl groups attached to the lipid classes used. Variation of the acyl groups gives different physicochemical properties, as will be seen in the Examples below.

The rate by which the Biosomes are formed from the BFM in a given aqueous environment can be affected and controlled by varying the geometrical shape of the main bilayer forming lipid class, i.e. the effective head group area in relation to the steric conformation of the hydrocarbon tails.

A second way of affecting and controlling the formation of lipid particles is by varying the structure, thus the fluidity, of the hydrocarbon chains in the nonpolar part of the lipid system. This will affect the rate of interaction of the endogenous amphiphatic lipids and the exogenous aqueous medium.

Careful selection of lipid constituents for a specific BFM is required in order to incorporate a bioactive compound *in vitro* and to achieve a carrier system which can deliver the bioactive compound, such as local anaesthetics, to the tissues through mucous membranes, through the skin or when applied directly to a wound. The lipid system is also chosen so that lipid particles can be formed in excess aqueous media. This involves the selection of lipid classes as well as the distribution of the fatty acid residues and therefore requires access to analytically pure and well characterized lipids.

Detailed description of the invention

According to the present invention the defined lipid system contains a drug which provides a systemic or local effect when administered transdermally or to mucous membranes or wounds. The drug is preferably a local anaesthetic such as lidocaine. The amount of the local anaesthetic is below 70 % (w/w) of the formulation, preferably below 50 % (w/w).

The BFM may also contain a given amount of a hydrophilic solvent, which can be water, glycerol, alcohols (e.g. ethanol), esters, or any mixture thereof. The amount in which the hydrophilic solvent is present is preferably low enough to

avoid phase separation. This limit depends on the physical nature of the components of the BFM and has to be established for each individual system.

In the lipid particle forming matrix the discrete particles are formed spontaneously from the matrix without any chemical or physical treatment or initiation.

When preparing the BFM the amphiphatic and polar lipid or the nonpolar lipid is mixed with the bioactive material *per se*, or in solution, and preferably the nonpolar lipid or lipids are admixed to the mixture of the bioactive material and the amphiphatic and polar lipid or lipids.

The lipid particle forming matrix as defined above may be used in pharmaceutical compositions such as topical, rectal, nasal, vaginal, buccal, ocular vehicles, creams, or ointments and they may also be used in the manufacture of a pharmaceutical composition for rectal, nasal, intravaginal, buccal, ocular administration or administration locally to the skin, to wounds or to mucous membranes.

In a preferred composition according to the invention the BFM, as defined above, is adapted for topical use by optionally adding suitable vehicles for administration to tissues by application to the skin, to wounds or to mucous membranes.

In another preferred composition according to the invention, the BFM, as defined above, is applied on a porous cellulose matrix, which is adhesive to mucous membranes. Other types of matrices adhesive to mucous membranes made from natural or synthetic solid or semi solid polymers are well-known to all those skilled in this art and will be equally useful for the purpose intended here.

Examples 1-7 below illustrate the variation of the lipid constituents of the BFM in the absence of drugs, by selection of lipids and combination thereof without limiting the scope of protection.

Examples 8-11 describe the preparation of formulations of BFMs that contain lidocaine with the use of lipid constituents from Examples 1-7 and how *in vivo* tests have been performed with a pin-prick test.

Example 12 describes a pin-prick test performed with a commercial lidocaine ointment, used as a reference for comparison with formulations prepared according to Examples 8-11.

Example 13 describes the preparation of a formulation of a BFM manufactured from commercially available lipids and lidocaine and the results of a pin-prick test of such a formulation.

Example 14 describes the preparation of a formulation of a BFM with lipid constituents chosen from Examples 1-7, which is applied on a cellulose matrix for administration to the buccal mucosa. The example shows also how to perform an *in vivo* test with the formulation.

Example 15 describes the preparation of a formulation according to Example 14 with commercially available lipids.

Table 1 presents formulations prepared according to Examples 8-11.

Table 2 presents the results of the pin-prick tests performed with the formulations according to Examples 8-12.

Table 3 presents the results of the pin-prick test performed with the formulation prepared according to Example 13 for comparison with the results in Table 2.

Table 4 presents formulations prepared as described in Examples 14-15 and the results of pin-prick tests performed with these formulations.

The incorporation of drugs, as illustrated by lidocaine, in the defined lipid system is surprisingly advantageous. The results show that drugs incorporated in formulations based on the BFM, i.e. the defined lipid system, can be absorbed very rapidly. This is illustrated by a fast and high anaesthetic effect when lidocaine is administered transdermally, compared to a commercial lidocaine ointment. The extremely rapid effect is also observed after buccal administration.

Furthermore, variations in the ratio between the well-defined lipid constituents can also be used to control the absorption rate, which is another important aspect of the invention.

Another method of controlling the absorption is to vary the chain length of the glycerides. A preferred chain length is between six and eighteen carbon atoms. A person skilled in the art can readily modify the inventive compositions in a manner to obtain formulations to suit individual purposes.

Various modifications and equivalents will be apparent to the person skilled in the art and may be used in the compounds, compositions and methods of the present invention without departing from the concept or scope thereof, and it is therefore to be understood that the invention is not limited to the specific examples and embodiments described herein.

EXAMPLES

EXAMPLE 1

1.25 g phospholipid from soybean (I) were added to 1.25 g of a glyceride mixture (II) and gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) were then added and the total mixture is stirred for 1 h at 60 °C.

<u>Lipid class composition (g)</u>	I	II	III	<i>Fatty acid composition of triacylglycerol (wt%)</i>	
Phosphatidylcholine	0.50				
Phosphatidylethanolamine	0.40				
Phosphatidylinositol	0.23				
Nonpolar lipids	0.12				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	58.5
				10:0 caprate	40.5
				12:0 laurate	0.6
				minors	0.4
Total	1.25	1.25	2.50	Total	100

EXAMPLE 2

1.25 g phospholipid from soybean (I) were added to 1.25 g of a glyceride mixture (II) and the mixture gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) were then added and the total mixture was stirred for 1 h at 60 °C.

<u>Lipid class composition (g)</u>	I	II	III	<i>Fatty acid composition of triacylglycerol (wt%)</i>	
Phosphatidylcholine	0.40				
Phosphatidylethanolamine	0.35				
Phosphatidylinositol	0.18				
Phosphatidic acid	0.07				
Nonpolar lipids	0.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				8:0 caprylate	58.5
				10:0 caprate	40.5
				12:0 laurate	0.6
				minors	0.4
Total	1.25	1.25	2.50	Total	100

EXAMPLE 3

1.25 g phospholipids from soybean (I) were added to 1.25 g of a glyceride mixture (II) and the mixture gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) were then added and the total mixture was stirred for 1 h at 60 °C.

<u>Lipid class composition (g)</u>	I	II	III	<i>Fatty acid composition of triacylglycerol (wt%)</i>	
Phosphatidylcholine	0.50				
Phosphatidylethanolamine	0.40				
Phosphatidylinositol	0.23				
Nonpolar lipids	0.12				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	10.0
				18:0 stearate	2.8
				18:1 oleate	20.6
				18:2 linoleate	58.9
				18:3 linolenate	6.7
				minors	1.0
Total	1.25	1.25	2.50	Total	100

EXAMPLE 4

1.25 g phospholipid from soybean (I) were added to 1.25 g of a glyceride mixture (II) and the mixture gently stirred for 12 h at 60 °C. 2.50 g of a triglyceride (III) were then added and the total mixture was stirred for 1 h at 60 °C.

<u>Lipid class composition (g)</u>	I	II	III	<i>Fatty acid composition of triacylglycerol (wt%)</i>	
Phosphatidylcholine	0.40				
Phosphatidylethanolamine	0.35				
Phosphatidylinositol	0.18				
Phosphatidic acid	0.07				
Nonpolar lipids	0.25				
Monoacylglycerol		0.63			
Diacylglycerol		0.63			
Triacylglycerol			2.50		
				16:0 palmitate	10.0
				18:0 stearate	2.8
				18:1 oleate	20.6
				18:2 linoleate	58.9
				18:3 linolenate	6.7
				minors	1.0
Total	1.25	1.25	2.50	Total	100

EXAMPLE 5

1.25 g phospholipid from soybean (I) were added to 1.25 g of a glyceride mixture (II) and the mixture gently stirred for 12 h at 60 °C.

<u>Lipid class composition (g)</u>	I	II
Phosphatidylcholine	0.40	
Phosphatidylethanolamine	0.35	
Phosphatidylinositol	0.18	
Phosphatidic acid	0.07	
Nonpolar lipids	0.25	
Monoacylglycerol		0.63
Diacylglycerol		0.63
Total	1.25	1.25

EXAMPLE 6

1.25 g phospholipid from soybean (I) were added to 1.25 g of a glyceride mixture (II) and 0.16 g ethanol. The total mixture was gently stirred for 6 h at 60 °C. 0.16 g of a triglyceride (III) was added and the total mixture was stirred for another hour at the elevated temperature.

<u>Lipid class composition (g)</u>	I	II	III
Phosphatidylcholine	0.40		
Phosphatidylethanolamine	0.35		
Phosphatidylinositol	0.18		
Phosphatidic acid	0.07		
Nonpolar lipids	0.25		
Monoacylglycerol		0.63	
Diacylglycerol		0.63	
Triacylglycerol			0.16
Total	1.25	1.25	0.16

EXAMPLE 7

2.50 g phosphatidylcholine from soybean (I) and 7.50 g of a monoglyceride (II) were gently stirred for 6 h at 60 °C. 1.25 g water were added and the stirring continued for a further hour at the elevated temperature.

<u>Lipid class composition (g)</u>	I	II	<i>Fatty acid composition of monoacylglycerol (wt%)</i>	
Phosphatidylcholine	2.50			
Monoacylglycerol		7.50		
			8:0 caprylate	79.6
			10:0 caprate	19.8
			12:0 laurate	0.2
			minors	0.4
Total	2.50	7.50	Total	100

EXAMPLE 8

Lipid formulation 1 was manufactured as follows:

7.75 g monoglyceride were heated to 60 °C and melted in a hot air oven. 1.75 g phosphatidylcholine were added and mixed gently until a homogeneous mixture was obtained at 60 °C. 0.50 g lidocaine base was finally added and mixed gently at 60 °C until a homogeneous mixture was obtained. The mixture was then placed at 25 °C and allowed to cool.

Pin-prick test for local anaesthetic effect was performed as follows:

The skin of the underside of the forearms was washed gently with a cotton wool cloth containing ethanol 70% (v/v). 1 g of formulation 1 was applied with a spatula on a surface 20 mm in diameter and covered by an occlusive silicone adhesive (Tegaderm®). Three application sites were used.

Formulation 1 was also tested diluted with equal parts of water immediately before application and in this case 2 g were applied on three test sites respectively and covered by the occlusive adhesive.

Four subjects was included in the test series and the two test formulations were removed after 0.5, 0.75 and 1.0 h. The test areas were dried with a dry paper cloth immediately after removal of the occlusive adhesive and the anaesthetic score was tested by pricking with a sterile injection cannula 10 times blind to the subject. The number of unregistered pricks was recoded and the anaesthetic score expressed as a percentage of the unregistered pricks.

EXAMPLE 9

The same procedure was applied as that in Example 8.

Formulation 2: Monoglyceride 6.00 g, phosphatidylcholine 3.50 g and lidocaine base 0.50 g.

Formulation 2:1: Diluted Formulation 2 with an equal part of water immediately before application.

Pin-prick test: Four (4) test areas of Formulations 2 and 2:1 respectively. Formulations removed after 0.50, 0.75, 1.00 and 2.00 h. Two (2) subjects.

EXAMPLE 10

The same procedure was applied as that in Example 8.

Formulation 3: Monoglyceride 5.00 g, phosphatidylcholine 3.50 g, water 1.00 g and lidocaine base 0.50 g.

Formulation 4: Monoglyceride 5.00 g, phosphatidylcholine 3.50 g, glycerol 1.00 g and lidocaine base 0.50 g.

Pin-prick test: Four (4) test areas of Formulation 3 and 4 respectively. Formulations removed after 0.50, 0.75, 1.00 and 2.0 h. Two (2) subjects.

EXAMPLE 11

The same procedure was applied as that in Example 8.

Formulation 5: Monoglyceride 1.00 g and triglyceride 5.00 g were melted together at 60 °C. Phosphatidylcholine 1.00 g added and finally lidocaine base 0.50 g.

Formulation 6: Monoglyceride 1.00 g and triglyceride 7.00 g were melted together at 60 °C. Phosphatidylcholine 1.50 g was added and finally lidocaine base 0.50 g.

Pin-prick test: Three (3) test areas of Formulation 5 and 6 respectively. Formulations removed after 0.50, 1.00, and 2.00 h. Pin-prick test was performed on the test area with 2.00 h application time also at 3.00, 4.00, 6.00, 8.00 and 10.00 h after initial application. Six (6) test subjects.

EXAMPLE 12

Formulation: Commercial lidocaine ointment 5% (Astra AB, Sweden).

Pin-prick test: 1.0 g applied on three (3) test areas. Six (6) test subjects. Formulation removed after 0.50, 1.00 and 2.00 h application time. Pin-prick test was also performed on the test site with 2 h application time also at 3.00, 4.00 and 6.00 hours after initial application.

EXAMPLE 13

Medium chain monoglyceride (Invitor®, Hüls) 6.00 g were melted at 60 °C in a hot air oven. Phosphatidylcholine 3.50 g (Sigma) were added and mixed gently. Lidocaine base 0.50 g was finally added and mixed gently for 5 hours at 60 °C.

Pin-prick test: Three (3) test areas. Formulation removed after 0.50, 1.00 and 2.00 h. Two (2) subjects.

EXAMPLE 14

Monoglyceride 1.00 g was melted together with triglyceride 5.00 g at 60 °C in a hot air oven until a homogeneous mixture was obtained. Phosphatidylcholine 3.50 g were then added and mixed gently to homogeneity. Finally lidocaine base 0.50 g was added and mixed gently until a homogeneous mixture was obtained.

A porous cellulose non-woven web (Wettex®, Teno AB, Sweden) 15 mm in diameter and 1 mm thick was soaked in the melted lipid formulation for 1 min and then allowed to cool at room temperature.

The cellulose web was administered to the inner chin mucosa on two (2) human volunteers and allowed to stay in contact with the buccal mucosa for 5 min. The web was then removed and local anaesthesia was tested by pricking with a sterile injection cannula 10 times. The number of unregistered pricks was recorded and the anaesthetic score expressed as the percentage of unregistered pricks.

EXAMPLE 15

Same description as Example 14 but with the commercial lipid raw materials. Two (2) subjects in the pin-prick test.

TABLE 1

LIPID FORMULATIONS WITH LIDOCAINE TESTED IN-VIVO (PIN-PRICK)

SUBSTANCE	FORMULATION %, (w/w)					
	1	2	3	4	5	6
LIDOCAINE BASE	5	5	5	5	5	5
MONOGLYCERIDE	77.5	60	50	50	10	10
PHOSPHATIDYLCHOLINE	17.5	35	35	35	35	15
TRIGLYCERIDE (MEDIUM CHAIN)	-	-	-	-	50	70
WATER	-	-	10	-	-	-
GLYCEROL	-	-	-	10	-	-

TABLE 2

PIN-PRICK TEST IN-VIVO (HUMAN)

RESULTS

TIME (h)	ANAESTHETIC SCORE FOR FORMULATION								
	REF	1	1:1*	2	2:1*	3	4	5	6
0	0	0	0	0	0	0	0	0	0
0.50	13	45	45	10	10	0	0	40	3
0.75	-	93	100	45	40	30	20	-	-
1.00	48	100	-	75	60	80	45	41	15
2.00	45**	-	-	100	85	100	95	40**	65**
3.00	48	-	-	-	-	-	-	37	63
4.00	15	-	-	-	-	-	-	35	43
6.00	0	-	-	-	-	-	-	19	-
8.00	-	-	-	-	-	-	-	9	17
10.00	-	-	-	-	-	-	-	0	0

*) Formulations 1 and 2 diluted with equal amount by weight of water . Two grams of diluted formulations 2 g were applied on the test area.

**) Removal of formulations after 2 h application following pin-prick testing performed on the same test site on the skin.

TABLE 3

PIN-PRICK TEST WITH LIPID FORMULATION MANUFACTURED WITH
COMMERCIAL LIPID QUALITIES

FORMULATION

Lidocaine base 5%, medium chain monoglyceride (Invitor®, Hüls) 60%,
phosphatidylcholine (Sigma) 35%.

RESULTS

<u>TIME (h)</u>	<u>ANAESTHETIC SCORE</u>
0	0
0.50	10
1.00	40
2.00	50

TABLE 4

BUCCAL ADMINISTRATION - LOCAL ANAESTHETICS

FORMULATIONS

SUBSTANCES	FORMULATION	
	1	2
LIDOCAINE BASE	5	5
MONOGLYCERIDE (ACCORDING TO THE INVENTION)	10	-
PHOSPHATIDYLCHOLINE (ACCORDING TO THE INVENTION)	35	-
TRIGLYCERIDE (ACCORDING TO THE INVENTION)	50	-
MONOGLYCERIDE (HÜLS)	-	10
PHOSPHATIDYLCHOLINE (SIGMA)	-	35
TRIGLYCERIDE (HÜLS)	-	50

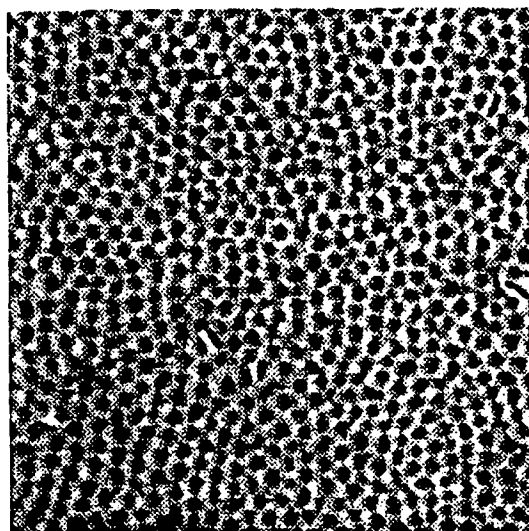
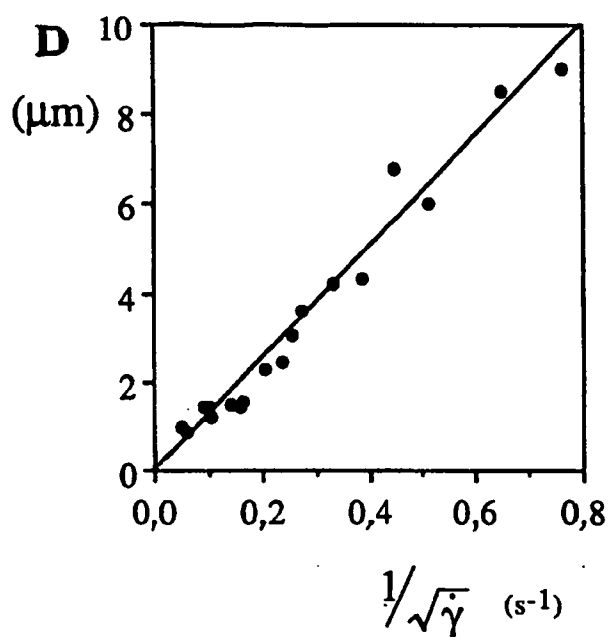
RESULTS

TIME (MIN)	ANAESTHETIC SCORE FOR FORMULATIONS	
	1	2
0	100	100
5	100	90
10	85	10
20	50	0
30	20	0

CLAIMS

1. A carrier system characterized in that it comprises a defined lipid system of at least two lipid components and at least one bioactive compound, wherein at least one of the lipid components is amphiphatic and polar and one is nonpolar, the bioactive compound is a local anaesthetic and the composition optionally contains a hydrophilic solvent.
2. System according to claim 1 characterized in that the hydrophilic solvent is water and/or glycerol.
3. System according to claims 1-2 characterized in that the amphiphatic and polar lipid components are bilayer forming and chosen from phospholipids and glycolipids in an amount of up to 80% w/w of the lipid system.
4. System according to claim 3 characterized in that the amphiphatic compound is a phosphatidylcholine and is present in an amount of 5-50% w/w of the lipid system.
5. System according to claims 1-4 characterized in that the nonpolar lipid component is chosen from the class of mono-, di- or triglycerides.
6. System according to claim 5 characterized in that the mono-, di-, or triglycerides have fatty acid components with chain lengths varying from six to eighteen carbon atoms.
7. System according to claim 5 characterized in that the nonpolar lipid component can consist of :
 - a) a triglyceride with essentially a mixture of 8:0 caprylate and 10:0 caprate and/or
 - b) a triglyceride with essentially a mixture of 18:2 linoleate, 18:1 oleate and 16:0 palmitate and/or
 - c) a monoglyceride with essentially a mixture of 8:0 caprylate and 10:0 caprate.

8. A pharmaceutical composition based on the system according to any of claims 1-7 characterized in that the system is combined with a solid or semi-solid polymer matrix, which can be applied on mucous membranes.
 9. A topical pharmaceutical composition for administration to tissues characterized in that the composition is based on the system according to any of the claims 1-7 and optionally other appropriate additives for application on the skin, on wounds or on mucous membranes.
 10. A pharmaceutical composition according to claims 8 or 9 characterized in that the local anaesthetic is lidocaine.
 11. Use of the carrier system according to any of claims 1-7 for manufacturing a pharmaceutical composition for transdermal administration or for administration locally on mucous membranes or on wounds.
-

$1/2$ FIG.1FIG.2

FEUILLE DE REMPLACEMENT

2/2

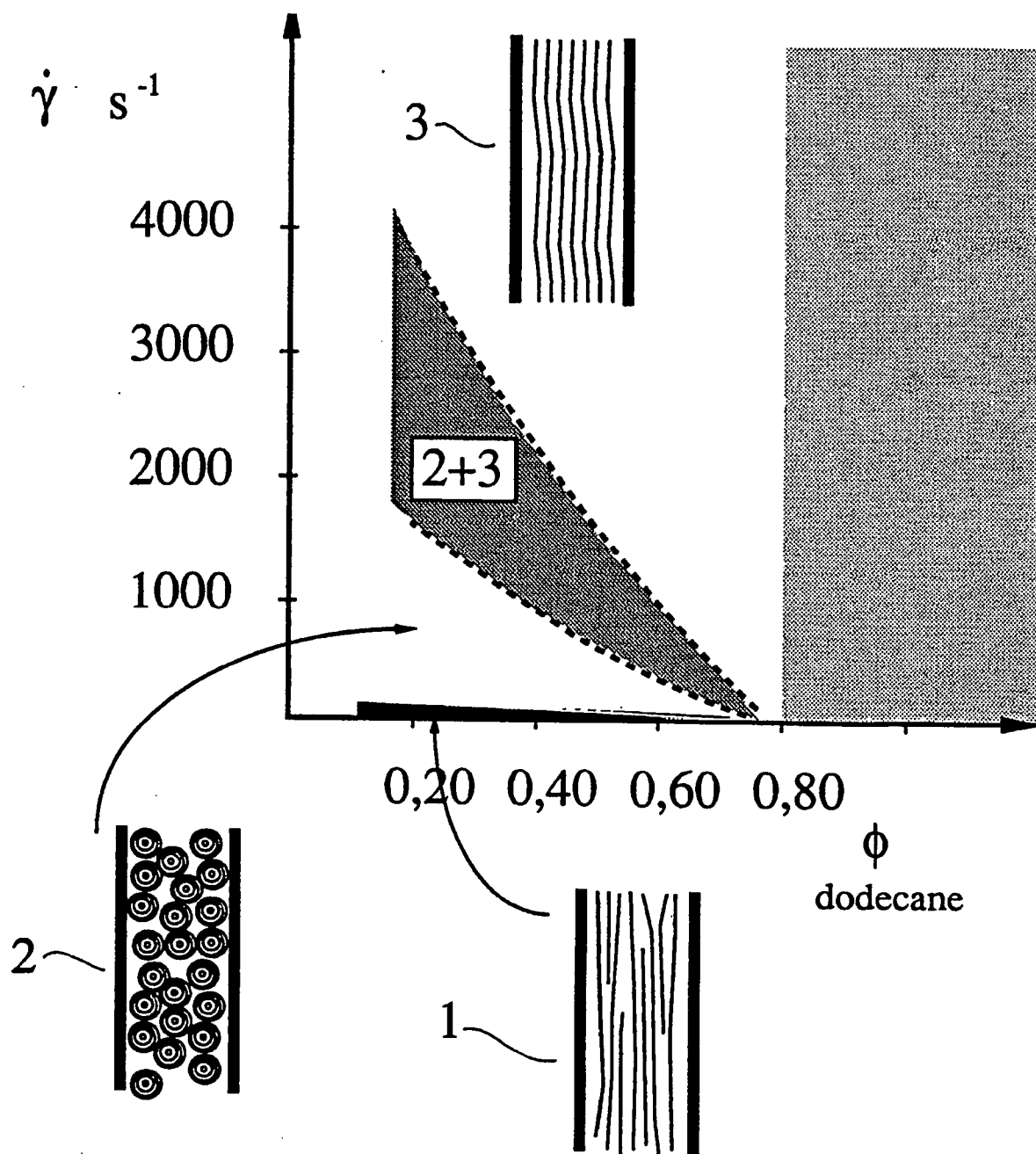


FIG. 3

FEUILLE DE REMPLACEMENT

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00257

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 9/127, A61K 47/44, B01J 13/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, WPIL, EMBASE, MEDLINE, CA, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO, A1, 9205771 (KABI PHARMACIA AB), 16 April 1992 (16.04.92) --	1-7
Y	EP, A2, 0158441 (PHARES PHARMACEUTICAL RESEARCH N.V.), 16 October 1985 (16.10.85), see page 5, line 1 - page 6, line 16; page 8, line 5 - page 9, line 18; page 12, line 18 - line 34 --	1-11
Y	WO, A1, 8707502 (PHARES PHARMACEUTICAL RESEARCH N.V.), 17 December 1987 (17.12.87), see pages 1-4, examples, claims --	1-11

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 93/00257

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>Dialog Information Services, file 154: MEDLINE 85-92/Dec, accession no. 07140017, Medline accession no. 90047017, Stozek T. et al: "The effect of entrapment of procaine hydrochloride in liposomes on local anesthetic action", & Pharmazie Jul 1989, 44 (7) p466-8</p> <p>-- -----</p>	1-11

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/93

International application No.
PCT/SE 93/00257

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9205771	16/04/92	AU-A- 8710591 EP-A- 0514506	28/04/92 25/11/92
EP-A2- 0158441	16/10/85	SE-T3- 0158441 DE-A- 3585967 JP-A- 61044808 US-A- 5004611 US-A- 5053217 US-A- 5141674	11/06/92 04/03/86 02/04/91 01/10/91 25/08/92
WO-A1- 8707502	17/12/87	DE-A- 3783039 EP-A,B- 0309464 SE-T3- 0309464	21/01/93 05/04/89

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- Published:
— With international search report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/01962 A1

(54) Title: A METHOD FOR THE IMPROVEMENT OF TRANSPORT ACROSS ADAPTABLE SEMI-PERMEABLE BARRIERS

(57) Abstract: The invention relates to a method, a kit and a device for controlling the flux of penetrants across an adaptable semi-permeable porous barrier, the method comprising the steps of: preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds of forms of amphiphilic substances with a tendency to aggregate; said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores, selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

5 A Method for the Improvement of Transport Across Adaptable Semi-
Permeable Barriers

The present invention is in the field of administration of drugs and particularly drug
delivery across barriers. It more particularly relates to a method for controlling the flux
10 of penetrants across an adaptable, semi-permeable porous barrier. It further relates to a
kit and a device which both enable the drug to be controllably applied.

A porous barrier as used herein is any obstacle comprising pores which are too narrow
to let the penetrants diffusively pass. This necessarily implies that the penetrants are
15 bigger than the average diameter of such a pore.

Some barriers, such as artificial porous membranes, for example ion-track
polycarbonate membranes, may have permanent properties, while others are
characterised by a possible change of their properties. Most notably the pore size and
20 more rarely the pore density, may change as a function of the surroundings and/or of
the flux of the penetrants through the pores in the barrier. The latter can be found with
living tissues which are separated by boundaries with such properties, for example,
cells and cell organelles.

25 The skin is used to further illustrate the basic principle of such a barrier:

The maximum barrier properties of the skin reside in the outermost skin region, that is,
in the horny layer (*stratum corneum*). This is owing to special chemical and
anatomical characteristics of the horny layer, which preclude most efficiently the
30 passage of essentially any material across the skin.

- 2 -

5 In the stratum corneum, 20-30 consecutive layers of the skin cells (chiefly corneocytes) are organised into columns. These columns are oriented perpendicular to the skin surface, permitting the cells from adjacent columns to overlap laterally and forcing the cells from one layer to be overlaid and packed densely. Intercellular junctions in the horny layer, moreover, are tightly sealed with specialised lipids, chiefly ceramides,
10 which abound in the skin. The skin lipids are also predominantly well packed: typically, they form lipid multilamellae, which are coupled covalently to the neighbouring cell (envelope) membranes. Individual multilamellar stacks that run parallel to the cells surface are joined together with the less well ordered lipid domains. In such domains, the non-ceramide lipids (fatty acids, cholesteryl-sulphate, etc.)
15 prevail.

The skin lipid tendency to self-arrange into densely packed, multilamellar structures is enhanced or even driven, by the hydration or certain ion (e.g. Ca^{2+}) concentration gradients in the skin. This may explain why similar lipid organisation is not observed
20 elsewhere in the body except, with a much lower abundance, in the oral cavity.

Chemical skin permeation enhancers, for example dimethylsulfoxide, promote the diffusion of drugs across the skin by solubilising or extracting some of the intercellular lipids from the barrier. Transcutaneous transport is therefore most efficient in the least
25 tightly packed lipid regions, where hydrophobic pores in the barrier are created most easily. Through such pores sufficiently small and lipophilic agents can diffuse along the transcutaneous concentration gradient(s). The resulting skin permeability is unaffected by the agent concentration, unless the agent acts as an enhancer, but the permeability depends on the concentration and the selection of skin permeation
30 enhancer(s).

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5 However the hydrophobic pores in the skin are not big enough to allow an appreciable transport of large drugs of any kind. Owing to the self-sealing tendency of the intercellular lipid domains the pores are also rather short lived. The lipophilicity of typical pores in the skin also precludes the transport of hydrophilic, that is, of highly polar, molecules across the organ. Conventional skin permeation enhancement is
10 therefore only useful for the delivery of fatty materials which do not irritate the skin too much, the enhancer-mediated transport and irritation being poorly tolerated by the consumers in many cases.

 Therefore to date, permeation based drug delivery through the skin is really
15 successful only for small drugs with a molecular weight below 400 Da. Such drugs can partition into the intercellular lipid matrix in the skin and then diffuse through small hydrophobic pores in the horny layer, first into the skin proper and then further down towards the deep body tissues. The resulting steady state transport is preceded by a short lag-time period, during which the drug traverses the barrier. Transcutaneous
20 transport does not suffer from the first pass effect, however.

 The bioavailability of drugs delivered through the skin by such conventional means is typically below 50 %, and often does not even reach 25 % (Hadgraft, 1996; Cevc, 1997).

25 Large hydrophobic molecules normally cross the skin in negligible quantity only. As already mentioned above this is due to the lack of suitable passages in the skin. Transcutaneous transport of macromolecules therefore chiefly relies on the molecular diffusion through shunts, such as pilosebaceous units. To deliver a bulky and highly
30 polar agent across the skin other methods than those conventionally used are therefore required. For example various skin poration techniques were introduced to create

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5 hydrophilic pores in the skin suitable for the purpose (to avoid confusion we will call such hydrophilic pores channels):

The simplest, and crudest solution, for making a wide channel through the skin is to eliminate mechanically the skin barrier. For example, to deliver a large, hydrophilic
10 antidiuretic peptide 1-deamino-8-D-arginine vasopressin across the human skin from an occlusive patch the removal of a small piece of epidermis by vacu-suction has been used (Svedman et al., 1996).

Further, a most common method for opening a wide channel through the skin is to use
15 an injection needle or mechanical impact(s) (injection; powderjection). Locally restricted skin challenge is also possible. This can be done by local heat application (thermoporation); by using high voltage pulses (>150 V; electroporation); or by acoustic energy, such as ultrasound (few $W\ cm^{-2}$; sonoporation). The resulting channel size depends on the nature and intensity of the skin treatment, but not on the nature or
20 the applied amount of molecules to be transported.

Openings or even craters in the skin created by the above mentioned methods heal rather slowly under normal application conditions; the wider the passage, the more so. The skin thus may behave as an adaptable, but slowly recoverable barrier.

25

Even the most commonly used methods for making pores in the skin rely on gadgets plus experience for the proper operation; they also involve skin disinfection to protect the patient. This notwithstanding, their harm and inconvenience is tolerated as long as therapeutic benefit is achieved.

30

- 5 -

5 The most recent tool for creating hydrophilic passages in those barriers, such as the skin is provided by microscopic barrier penetrants which directly and reversibly open said hydrophilic channels. Such penetrants are independent of external energy source and also do not rely on any gadgets. They are also well tolerated by the skin. Such penetrants known to date all belong to the class of highly deformable complex
10 droplets (Transfersomes®). Such droplets adapt to the pores of the barrier - which they then cross efficiently - provided that the droplet components and preparation are properly selected and/or optimised. A sufficiently adaptable and hydrophilic droplet can therefore cross the barrier, such as skin, spontaneously. Such hydrophilic channels are opened transiently by the moving penetrant after the latter has adjusted its shape to
15 achieve the goal. This allows the adjustable droplets to act as vehicles for the delivery of various - hydrophilic or hydrophobic - agents across the barrier.

Most useful droplets comprise an aqueous core surrounded by an highly flexible mixed lipid bilayer, which makes the aggregate ultradeformable and superficially highly
20 hydrophilic. Both is required for an efficient transcutaneous transport (Cevc, 1997). Said droplets were demonstrated to transport their mass rather efficiently across the skin under optimum application conditions (Cevc, 1997).

Other types of aggregates (liposomes, niosomes, nanoparticles, microemulsions, etc.)
25 also have been claimed to traverse the skin efficiently but were seldom, if ever, proven really to deliver the associated drugs across the skin in practically meaningful quantities. It is believed that in contrast to the highly deformable droplets (Transfersomes®) the used aggregates are either insufficiently deformable and/or are too unstable to achieve the goal. Conventional aggregates instead act as simple drug
30 reservoirs on the skin: the aggregates, incapable of crossing the barrier, remain on the skin while the drug is released gradually from the 'vehicle' to then probably diffuse

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5 through the skin barrier on its own. The main action of conventional drug loaded suspensions is thus to increase the skin barrier hydration and/or to shed the molecules with the skin permeation enhancing capability into the tissue.

Contrary, the composite, ultradeformable lipid droplets (Transfersomes®) deform and
10 then penetrate the skin rather than to coalesce locally. Such aggregates motion across the skin seems to proceed along the natural moisture gradient(s) between the skin cells, which guides the aggregates into the hydrophilic (virtual) channels in the organ.

The predecessors of those channels that let highly adaptable droplets pass through the
15 skin are originally so narrow that they only permit evaporation of (rather small) water molecules across the skin. These originally tiny pores (diameter < 0.5 nm) seem to open reversibly, however, when the stress of partial dehydration of a droplet, which is thereby being forced into the channel mouth under non-occlusive conditions, becomes excessive. The strong hydrophilicity and the large mass of the droplet are the factors
20 which maximise the droplets' tendency to move through the skin; however the droplet adaptability is the necessary condition for the success of said motion.

The movement of the droplets across the skin seems to proceed along the path pursued by the water molecules during the skin passage in the opposite direction. The droplets
25 are thus guided into intercellular regions precisely at the points where the contacts between the above-cited skin sealing lipids are the weakest and the least tight. The corresponding skin region covered with the channels has been estimated to be around 4 % of the total skin area, or less.

30 It is possible to associate small and large, hydrophobic and hydrophilic molecules with ultradeformable and highly adaptable droplet-like aggregates. Using such complex

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- 5 aggregate droplets all types of molecules can thus be delivered across the barrier, such as the stratum corneum.

High systemic availabilities of the drug transported are typically achieved. Relative efficiency of the transport across the skin exceeds 50 %, in most cases (Cevc et al.,
10 1996). The steady state is reached within few hours, by and large (Cevc et al., 1998).

It has already been proven that the skin barrier recovers fully after those droplets have been eliminated from the skin surface. In contrast, the channels created by other means, such as ultrasound remain open for at least 20 hours. In fact, they are not resealed
15 properly before 2 days, even when relatively weak therapeutic ultrasound is used. Stronger perturbation causes more persistent skin damage (Mitragotri et al., 1995). (In the extreme case, when the barrier is eliminated by vacu-suction, the skin does not recover fully until after of 8 weeks.)

- 20 The precise size distribution of the channels in the skin, through which highly deformable droplets migrate spontaneously across the stratum corneum, is as yet unknown. It is probable, however, that it is asymmetric. The average width, that is, the distribution maximum has been estimated to be 20-30 nm under typically used application conditions. The skewed distribution could result from the existence of two
25 quantitatively different but qualitatively similar intercellular transport routes across the skin (Schätzlein & Cevc, 1998) which together form the family of transcutaneous pathways.

The first, inter-cluster pathway leads between the groups of corneocytes. It represents
30 the high-end tail of channel-size distribution and typically starts at the bottom of inter-

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5 cluster gorges. From here, it follows the dense material filling such gorge and offers the lowest resistance to penetration at the junctions where several clusters meet.

The second, intra-cluster pathway leads between the individual corneocytes in each cluster of corneocytes. This route typically proceeds along the lipid layers surface. In
10 the projection over the outer third of the stratum corneum, the inter-corneocyte pathway resembles an interwoven three-dimensional network including all the cells in the organ. (Schätzlein & Cevc, 1998).

The above mentioned distinctions are quantitative in nature. No doubt exists that
15 transcutaneous channels with the exception of pilosebaceous units are resistant to the passage of non-deformable, large aggregates.

Channel properties are also sufficiently constant to reveal little inter-site, inter-individual, inter-species or inter-carrier variability. According to the prior art, the
20 relative bio-availability of different drugs in the blood after an epicutaneous administration in highly adaptable droplets (Transfersomes®) is fairly constant (Cevc, 1997). Pore distribution depends little on the nature of the penetrant or the drug. The same has been implied for the dose dependence, which was concluded to affect merely the depth of penetrant and drug distribution. Small dose per area was found to favour
25 the local (superficial) retention whereas a large dose per area was shown to ensure a relatively great systemic availability.

Surprisingly, and contrary to the above-mentioned conclusion, we have now found out that changing the applied dose above a certain threshold and in sufficiently wide range
30 not only affects the drug/penetrant distribution, but also determines the rate of penetrant transport across the barrier.

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- 5 Our new and unexpected finding provides means for controlling the rate of transcutaneous drug delivery whenever highly deformable carriers are used on the barrier; it also provides the basis for better, i.e. more rational, design of the delivery device. There will especially be profit for the development of cutaneous patches suitable for the use in combination with highly adaptable carriers (Transfersomes®).
- 10 Improved therapy and higher commercial value of the products should be the consequence.

It stands to reason that the observed new effect reflects the widening of channels in the barrier, but the applicant does not wish to be bound to this hypothesis. The newly
15 found dosage-dependent pore widening is probably different for various transcutaneous channels: the originally narrower pores probably change more than the relatively wide (e.g. inter-cluster) channels. The effect of relative channel size, that is, of channel vs. penetrant size ratio, suggests that it will take much longer time to bring certain penetrants quantity through narrow than through wide channels.

- 20 If the channels act as transported mass discriminators, and adjust their width to the flux requirement, the narrow channels will persist much longer in their original, high penetration resistance state than the wide channels. However, after having responded to the multi-penetrant passage by increasing their width such channels will start to
25 behave as the originally wider channels. Multiple adjustments are possible but only to certain upper limit.

- Another potentially important factor acting in the same direction is the skin surface hydration, which is prone to increase with enlargement of the topically administered
30 dosage. Similar mechanism has been proposed to explain the effect of conventional lipid suspension (liposomes) on the diffusive transport of large entities into the skin

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5 (Cevc, 1997). In either case, the average width and the size distribution of channels in the skin will shift towards greater values with increasing applied dosage. This then will result in higher final transcutaneous flux.

For the avoidance of doubt, all pertinent information, definitions and lists from the
10 previous patent applications of the same applicant are incorporated herein by reference.

Kits and more particularly devices for administering drugs through a barrier such as skin or mucosa have also already been described. These devices can typically be divided into matrix systems and liquid reservoir systems. They
15 are commonly in the form of a laminated composite that includes a reservoir layer containing the drug, a pressure sensitive adhesive layer for attaching the composite to the skin, and a backing layer that forms the upper layer of the device. Depending upon the particular drug and drug formulation involved, the reservoir layer may be a matrix in which the drug formulation
20 is dispersed or a layer in the form of walled container which holds the drug formulation. Container-type reservoirs are often formed as a pocket between the backing layer and a drug-permeable basal membrane through which the drug passes to the skin. The pressure sensitive adhesive layer normally underlies the membrane and the drug also passes through it on its way to the
25 skin.

Matrix-type transdermal patches are those in which the drug is contained in and released from a polymer matrix. The matrix is typically made of a pressure sensitive adhesive and defines the basal surface of the patch (i.e.
30 the surface affixed to the skin).

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5 A number of reservoir and matrix type systems have been described.

US-Patent No. 829,224 to Chang et al., for instance, discloses a device with a reservoir that is defined by a backing layer and a drug-permeable membrane layer. A ring-shaped layer made of an adhesive is peripheral to the reservoir. A peelable liner layer underlies the membrane. A second peelable layer, the release liner, underlies the entire assembly. A first heat seal connects the backing layer and the membrane and surrounds the reservoir. A second heat seal concentric about the first heat seal connects the backing layer and the release liner. The second heat seal is broken when the release liner is removed. The device may include an inner liner that underlies the membrane and portions of the backing layer. This inner liner is removed following removal of the release liner so that the membrane is exposed.

20 U.S.-Patent Nr. 4,983,395 to Chang et al., relates to another device with a backing layer and a membrane layer that define a reservoir. A peelable inner liner underlies the reservoir and portions of the backing and membrane layers outside the periphery of the reservoir. An adhesive layer underlies the inner liner and remaining portions of the backing and membrane layers. A peelable release liner underlies the adhesive layer. A first heat seal connects the backing and membrane layers on the periphery of the reservoir. A second heat seal underlies the first heat seal and connects the membrane and the inner liner. In use, the release liner and inner liner are peeled away to expose the undersurfaces of the membrane and adhesive layers prior to placement of the device onto the skin or mucosa.

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5 PCT-Application W096-19205 to Theratech, Inc., discloses a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of an adhesive overlay, a backing layer underlying the central portion of the adhesive overlay, an active agent-permeable membrane, the backing layer and membrane defining a reservoir
10 that contains a formulation of the active agent, a peel seal disc underlying the active agent-permeable membrane, a heat seal about the periphery of the peel seal disc, the active agent-permeable membrane and the backing layer and a removable release liner underlying the exposed overlay and peel seal disc. The adhesive layer is above and peripheral to the path of the active
15 agent to the skin or mucosa and is protected from degradation by the components of the reservoir by a multiplicity of heat seals. The peel seal disc protects against release of the active agent-containing reservoir and the release liner protects the adhesive from exposure to the environment prior to use.

20 US-Patent No. 5,202,125 to Theratech, Inc., describes a transdermal delivery system for delivery of nitroglycerin which deliver the drug at enhanced transdermal fluxes. The systems include, in addition to nitroglycerin, a permeation enhancer which is either a sorbitan ester, a C8-
25 C22 aliphatic alcohol, or a mixture thereof. Methods for administering nitroglycerin using such permeation enhancers are also disclosed.

WO90-11065 to Theratech, Inc., discloses a transdermal drug delivery device comprising a drug formulation containing reservoir defined by a
30 backing layer and a drug-permeable membrane layer, a peelable inner liner that underlies the reservoir and a portion of the backing/membrane

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5 outwardly of the reservoir periphery, an adhesive layer that underlies the inner liner and outwardly extending portions of the membrane/backing layers, and a peelable release liner layer that underlies the adhesive layer with a first permanent heat seal between the backing and the membrane about the perimeter of the reservoir and another peelable (impermeant) heat
10 seal between the membrane and the inner liner underlying the first permanent heat seal, the heat seals and peelable barrier layer providing barriers that isolate the drug formulation from the adhesive.

US-Patent No. 5,460,820 to Theratech, Inc., discloses a method of providing
15 testosterone replacement therapy to a woman in need of such therapy comprising applying a testosterone-delivering patch to the skin of said woman which patch transdermally delivers 50 to 500 µg/day testosterone to the woman. The skin patch comprises a laminated composite of a backing layer and a matrix layer comprising a solution of testosterone in a polymeric
20 carrier, said matrix layer providing a sufficient daily dose of testosterone to provide said therapy.

US-Patent No. 5,783,208 to Theratech, Inc., discloses a matrix-type transdermal patch for coadministering estradiol and another steroid wherein
25 the matrix is composed of a N-vinyl-2-pyrrolidone-containing acrylic copolymer pressure sensitive adhesive, estradiol the other steroid, and optionally a permeation enhancer, and the respective fluxes of estradiol and the other steroid from the matrix are independent of the respective concentrations of the other steroid and estradiol in the matrix.

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- 5 All pertinent information, definitions and lists from the patents and patent applications of the US-company Theratech, Inc. are expressively incorporated herein by reference.

10 It is an important object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier, such as the skin of a human or animal body or a plant. It is another object of the present invention to control the flux of highly deformable penetrants (Transfersomes®) across an adaptable semi-permeable porous barrier in using a kit or device which enables the
15 formulation to be applied at the selected dose per area.

According to the present invention this is achieved by a method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:

- 20 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
- 25 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
- and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the
30 diameter of homo-aggregates containing merely the less soluble substance,

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- 5 – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher;
- 10 – and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- 15 – said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- 20 – selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- 25 – applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

Preferrably the flux of penetrants across said barrier is increased by enlarging the applied dose amount of said penetrants.

25

It then is preferred if the pH of the formulation is between 3 and 10, more preferably is between 4 and 9, and most preferably is between 5 and 8.

According to another preferred feature of the present invention the
30 formulation containing the penentrants comprises:

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- 5 – at least one thickening agent in an amount to increase the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- 10 – and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per 12 months
- 15 – and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 4 days.

It then is preferred if said at least one microbicide is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass

20 of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphilococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

25 It then is also preferred if said thickening agent is selected from the class of pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates,

30 poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-

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- 5 sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol-lactides, polyethylene glycol-diacrylates, polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides), poly(propylene fumarate-co-ethylene glycols), poloxamers, polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;
- 10 natural gums comprising alginates, carrageenans, guar-gums, gelatines, tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses; mixtures and further derivatives or co-polymers thereof and / or other pharmaceutically, or at least biologically, acceptable polymers.
- 15 Preferably the concentration of said polymer is chosen to be in the range between 0.01 w- % and 10 w- %, more preferably in the range between 0.1 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.
- 20 Further it is preferred that said anti-oxidant is selected from synthetic phenolic antioxidants, such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-
- 25 trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid , salicylic acid, nordihydroguaiaretic
- 30 acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.

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- 5 -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.);
- 10 non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital,
- 15 acetaminophen); aminosalicylic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium
- 20 metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less
- 25 complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-)
- 30 chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol,

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5 carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid;
oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers,
sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives
(e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine,
3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation
10 suppressing enzyme.

Then, the concentration of BHA or BHT is often chosen to be between
0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most
preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between
15 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most
preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005
and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most
preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between
0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably
20 is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5,
more preferably is between 0.005 and 0.5 w-%, and most preferably is
between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is
between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and
most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001
25 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably
is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is
between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most
preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of
monothioglycerol is between 0.01 and 5 w-%, more preferably is between
30 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically
0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between

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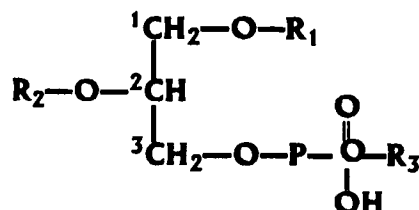
- 5 0.001–0.2 w-%, and most preferably is between 0.005–0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most
10 preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01–0.2, most typically between 0.3 and 2 w-%.
- 15 Furthermore it is preferred if said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially
20 alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide,
25 cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.
- 30 Preferrably the bulk concentration of short chain alcohols in the case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up

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- 5 to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the
10 range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.
- 15 It is preferred that the less soluble amongst the aggregating substances is a lipid or lipid-like material, especially a polar lipid, whereas the substance which is more soluble in the suspending liquid and which lowers the average elastic energy of the droplet is a surfactant or else has surfactant-like properties and / or is a form of said lipid or lipid-like material which is
20 comparably soluble as said surfactant or the surfactant-like material.

Preferably the lipid or lipid-like material is a lipid or a lipoid from a biological source or a corresponding synthetic lipid or any of its modifications, said lipid preferably belonging to the class of pure
25 phospholipids corresponding to the general formula

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5

where R_1 and R_2 is an aliphatic chain, typically a C_{10-20} -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-,
 10 myristoyl-, palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also
 15 glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids,
 20 phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-
 25 groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

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- 5 The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-
- 10 aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
- 15 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-
- 20 lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-
- 25 or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-
- 30 glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,

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- 5 or -phosphorylserine, n-tetradecyl- glycerophosphatidic acid, -
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain
phospholipid, or else a surface-active polypeptide.
- 10 According to a preferred feature of the present invention, the average
diameter of the penetrant is between 30 nm and 500 nm, more preferably
between 40 nm and 250 nm, even more preferably between 50 nm and
200 nm and particularly preferably between 60 nm and 150 nm.
- 15 It is another preferred feature of the present invention that the total dry
weight of droplets in a formulation is 0.01 weight-% (w-%) to 40 w-% of
total formulation mass, more preferably is between 0.1 w-% and 30 w-%,
and most preferably is between 0,5 w-% and 20 w-%.
- 20 It is preferred that the total dry weight of droplets in a formulation is
selected to increase the formulation viscosity to maximally 5 Nm/s, more
preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that
formulation spreading-over and drug retention at the application area is
enabled.
- 25 According to the present invention is preferred if at least one edge-active
substance or surfactant and/or at least one amphiphilic substance, and / or at
least one hydrophilic fluid and the agent are mixed, if required separately, to
form a solution, the resulting (partial) mixtures or solutions are then
- 30 combined subsequently to induce, preferably by action of mechanical energy
such as shaking, stirring, vibrations, homogenisation, ultrasonication,

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5 shearing, freezing and thawing, or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

Preferrably this amphiphilic substances are dissolved in volatile solvents,
10 such as alcohols, especially ethanol, or in other pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed,
15 especially by evaporation or dilution, prior to making the final preparation.

According to the present invention the formation of said penetrants
preferrably is induced by the addition of required substances into a fluid
phase, evaporation from a reverse phase, by injection or dialysis, if
20 necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

25 Then the formation of said penetrants preferrably is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

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- 5 According to the invention said agents and penetrants preferably are made to associate, at least partly,
- after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol
 - 10 (molecular weight: 200-400 D) or glycerol into the suspending medium,
 - simultaneously with penetrant formation, if required using the drug co-solution and, at least some, penetrant ingredients.

It is preferred if said penetrants, with which the agent is associated are
15 prepared immediately before the application of the formulation, if convenient, from a suitable concentrate or a lyophilisate.

The formulation according to the invention preferably is applied by spraying, smearing, rolling or sponging on the application area, in particular
20 by using a metered sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

It is preferred if the barrier is a part of a mammalian body and / or a plant and preferably is skin and / or at least partly keratinised endothelium
25 and / or nasal or any other mucosa.

The area dose of said penetrant then preferably is between 0.1 mg per square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between
30 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on said skin and / or said at least partly keratinised endothelium.

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5 The area dose of said penetrant then preferably is between 0.0001 mg per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is applied on plant body, plant leaves or plant needles.

10

The area dose of said penetrant then preferably is between 0.05 mg per square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on

15 said nasal or other mucosa.

In another advantageous aspect of the invention, a kit containing said formulation in an amount which enables the formulation to be applied at the selected dose per area as afore-mentioned is provided.

20

It then is preferred if the formulation is contained in a bottle or any other packaging vessel.

The kit preferably contains a device for administering the formulation,
25 which preferably is a non-occlusive patch.

According to another aspect of the present invention a device is provided comprising a non-occlusive patch, containing the formulation in an amount that yields the dose per area as mentioned above.

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- 5 According to the invention a non-occlusive patch comprises a laminated composite of:
- a backing layer;
 - an active agent-permeable membrane, the backing layer and membrane defining
 - 10 – a reservoir therebetween that contains the formulation of the active agent, said reservoir having a smaller periphery than the backing layer and membrane such that a portion of the backing layer and membrane extends outwardly of the periphery of the reservoir;
 - a pressure sensitive adhesive layer that underlies and covers the active
 - 15 agent-permeable membrane and said outwardly extending portion of the backing layer and membrane.

According to the invention a non-occlusive patch also comprises a laminated composite of:

- 20 – a backing layer;
- a matrix layer that contains the formulation of the active agent; and
 - a pressure sensitive adhesive layer.

It then is preferred if the formulation and / or agent and / or

25 suspension / dispersion of penetrants without the agent are kept during the storage in several, more preferably less than 5, even more preferably in 3, and most preferred in less than 3 separate inner compartments of the device which, in case, are combined prior to or during the application of the formulation.

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- 5 Preferably said compartment(s) filled with the formulation and / or agent and / or suspension of penetrants without the agent, is (are) covered, on one or both sides, with a non-occlusive, semi-permeable membrane that lets small molecules, such as water, but only few or not the penetrants pass.
- 10 It is preferred that said non-occlusive, semi-permeable membrane is the same or different, if it is used on both sides of said device.

Accordingly it is preferred if said water permeability of said semi-permeable membrane is at least $10 \text{ mg cm}^{-2} \text{ h}^{-1}$, more preferably exceeds $50 \text{ mg cm}^{-2} \text{ h}^{-1}$

15 and most preferably is greater than $100 \text{ mg cm}^{-2} \text{ h}^{-1}$.

The area of said semi-permeable membrane preferably is between 0.5 cm^2 and 250 cm^2 , more preferably is between 1 cm^2 and 100 cm^2 , even more preferably is between 2 cm^2 and 50 cm^2 and most preferred is between 4 cm^2

20 and 25 cm^2 .

It is preferred if the area of said semi-permeable membrane is the area substantially covered by the formulation filled part of the device.

- 25 It further is preferred if the penetrant flux across the barrier is controlled by the permeability of, or the suspension-medium evaporation across, the semi-permeable, non-occlusive membrane.

According to a preferred feature of the invention the device is filled with the

30 formulation and / or agent molecules and / or suspension of penetrants without agent, either separately or together, prior to the administration of

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5 said patch, preferably 360 min, more preferably 60 min, even more
 preferably 30 min and most preferably within few minutes before placing
 the device on the barrier.

 It another important aspect of the present invention, a method is provided of
10 administering an agent onto a mammalian body or a plant, for transporting
 said agent through a barrier, such as the intact skin/mucosa or cuticle,
 respectively, when the agent is associated with the penetrant which is
 capable of transporting said agent through the skin pores or through the
 passages in mucosa or cuticle, or else is capable of enabling agent
15 permeation through skin pores after said penetrant has opened and/or
 entered said pores, comprising the steps of:

- preparing a formulation by suspending or dispersing said penetrants in a
 polar liquid in the form of fluid droplets surrounded by a membrane-like
 coating of one or several layers, said coating comprising at least two
20 kinds or forms of amphiphilic substances with a tendency to aggregate,
 provided that
- said at least two substances differ by at least a factor of 10 in solubility in
 said polar liquid,
- and / or said substances when in the form of homo-aggregates (for the
25 more soluble substance) or of hetero-aggregates (for any combination of
 both said substances) have a preferred average diameter smaller than the
 diameter of homo-aggregates containing merely the less soluble
 substance,
- and / or the more soluble substance tends to solubilise the droplet and the
30 content of such substance is to up to 99 mol-% of solubilising

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- 5 concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
- and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more
 - 10 than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
 - said penetrants being able to transport agents through the pores of said barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,
 - 15 – selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
 - applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

20

It then is preferred if the flux across said barrier is increased by enlarging the applied dose amount of said penetrants per area of barrier.

The pH of the formulation preferably is chosen to be between 3 and 10,
25 more preferably is between 4 and 9, and most preferably is between 5 and 8.

In this aspect of the invention, it then is preferred if the formulation comprises:

- at least one thickening agent in an amount to increase the formulation
- 30 viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most

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- 5 preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,
- and / or at least one antioxidant in an amount that reduces the increase of oxidation index to less than 100 % per 6 months, more preferably to less than 100 % per 12 months and most preferably to less than 50 % per
- 10 12 months
- and / or at least one microbicide in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 4 days.
- 15

Said at least one microbicide then preferably is added in an amount that reduces the bacterial count of 1 million germs added per g of total mass of the formulation to less than 100 in the case of aerobic bacteria, to less than

20 10 in the case of entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days, and more preferably after a period of 1 day.

Said thickening agent preferably is selected from the class of

25 pharmaceutically acceptable hydrophilic polymers, such as partially etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers such as polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-,

30 poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,

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- 5 polyethylene glycol-lactides, polyethylene glycol-diacrylates,
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),
poly(propylene fumarate-co-ethylene glycols), poloxamers,
polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;
natural gums comprising alginates, carrageenans, guar-gums, gelatines,
10 tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;
mixtures and further derivatives or co-polymers thereof and / or other
pharmaceutically, or at least biologically, acceptable polymers.

- The concentration of said polymer then preferably is chosen to be in the
15 range between 0.01 w- % and 10 w- %, more preferably in the range
between 0.1 w- % and 5 w- %, even more preferably in the range between
0.25 w- % and 3.5 w- % and most preferably in the range between 0.5 w- %
and 2 w- %.

- 20 According to the invention said anti-oxidant then preferably is selected
from synthetic phenolic antioxidants, such as butylated hydroxyanisol
(BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol
(LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-5119,
BI-L-239XX, etc.), tertiary butylhydroquinone (TBHQ), propyl gallate
25 (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines
(such as diphenylamine, p-alkylthio-o-anisidine, ethylenediamine
derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids
(such as guaiacol, hydroquinone, vanillin, gallic acids and their esters,
protocatechuic acid, quinic acid, syringic acid, ellagic acid , salicylic acid,
30 nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including
tocopherols (alpha, beta, gamma, delta) and their derivatives, such as

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5 tocopheryl-acylate (e.g. -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or
10 linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine,
15 azathioprine, phenobarbital, acetaminophen); aminosalicic acids and derivatives; methotrexate, probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol);
20 sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase,
25 glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters,
30 caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace,

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5 oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic
10 acid), and organochalcogenides, or else is an oxidation suppressing enzyme. It then is preferred if the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most
15 preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5,
20 more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably
25 is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically
30 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most

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5 typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and
10 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

Preferrably said microbicide is then selected amongst short chain alcohols,
15 such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben,
20 benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as
25 phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibioticly active compounds of biological origin, or a mixture thereof.

It then is preferred that the bulk concentration of short chain alcohols in the
30 case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between

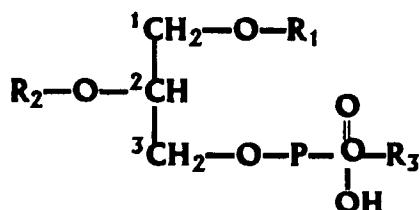
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5 0.5-3 w-%, and in the case of chlorobutanol is in the range between
0.3-0.6 w-%; bulk concentration of parabens, especially in the case of
methyl paraben is in the range between 0.05-0.2 w-%, and in the case of
propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration
of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of
10 benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of
phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk
concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

It then is also preferred that the less soluble amongst the aggregating
15 substances is a lipid or lipid-like material, especially a polar lipid, whereas
the substance which is more soluble in the suspending liquid and which
lowers the average elastic energy of the droplet is a surfactant or else has
surfactant-like properties and / or is a form of said lipid or lipid-like material
which is comparably soluble as said surfactant or the surfactant-like
20 material.

Preferrably the lipid or lipid-like material is a lipid or a lipoid from a
biological source or a corresponding synthetic lipid or any of its
modifications, said lipid preferably belonging to the class of pure
25 phospholipids corresponding to the general formula

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5

where R_1 and R_2 is an aliphatic chain, typically a C_{10-20} -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-,
 10 myristoyl-, palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also
 15 glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids,
 20 phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-
 25 groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

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- 5 The surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-
- 10 aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
- 15 ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-
- 20 lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-
- 25 or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-
- 30 glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,

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- 5 or -phosphorylserine, n-tetradecyl- glycerophosphatidic acid, -
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain
phospholipid, or else a surface-active polypeptide.
- 10 The average diameter of the penetrant preferably is between 30 nm and
500 nm, more preferably between 40 nm and 250 nm, even more preferably
between 50 nm and 200 nm and particularly preferably between 60 nm and
150 nm.
- 15 The total dry weight of droplets in a formulation is then preferably chosen
to range from 0.01 weight-% (w-%) to 40 w-% of total formulation mass,
more preferably is between 0.1 w-% and 30 w-%, and most preferably is
between 0,5 w-% and 20 w-%.
- 20 Preferably the total dry weight of droplets in a formulation is selected to
increase the formulation viscosity to maximally 5 Nm/s, more preferably up
to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation
spreading-over and drug retention at the application area is enabled.
- 25 Preferably at least one edge-active substance or surfactant and/or at least
one amphiphilic substance, and / or at least one hydrophilic fluid and the
agent are mixed, if required separately, to form a solution, the resulting
(partial) mixtures or solutions are then combined subsequently to induce,
preferably by action of mechanical energy such as shaking, stirring,
30 vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,

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5 or filtration using convenient driving pressure, the formation of penetrants that associate with and / or incorporate the agent

It also is preferred if said amphiphilic substances then are dissolved in volatile solvents, such as alcohols, especially ethanol, or in other
10 pharmaceutically acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the
15 final preparation.

The formation of said penetrants then preferably is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical
20 stress, such as shaking, stirring, especially high velocity stirring, vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

It then is also preferred if the formation of said penetrants is induced by
25 filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used sequentially or in parallel.

30 Said agents and penetrants are made to associate, at least partly,

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- 5 – after the formation of said penetrants, e.g. after injecting a solution of the
 drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and
 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol
 (molecular weight: 200-400 D) or glycerol into the suspending medium,
 – simultaneously with penetrant formation, if required using the drug
10 co-solution and, at least some, penetrant ingredients.

It then is preferred if said penetrants, with which the agent is associated, are
prepared immediately before the application of the formulation, if
convenient, from a suitable concentrate or a lyophilisate.

15

Accordingly the formulation is applied by spraying, smearing, rolling or
sponging on the application area, in particular by using a metered sprayer,
spender, roller or a sponge, or a non-occlusive patch, as appropriate.

- 20 It further is preferred if the barrier is skin or at least partly keratinised
 endothelium and / or nasal or any other mucosa.

- The area dose of said penetrant then preferably is between 0.1 mg per
square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between
25 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between
 0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on
 said skin and / or said at least partly keratinised endothelium.

- The area dose of said penetrant preferably is between 0.05 mg per square
30 centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between
 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between

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- 5 0.5 mg cm⁻² and 10 mg cm⁻², in the case that the penetrant is applied on said nasal or other mucosa.

- The area dose of said penetrant preferably is between 0.0001 mg per square centimetre (mg cm⁻²) and 0.1 mg cm⁻², more preferably is between
10 0.0005 mg cm⁻² and 0.05 mg cm⁻² and even more preferably is between 0.001 mg cm⁻² and 0.01 mg cm⁻², in the case that the penetrant is applied on plant body, plant leaves or plant needles.

- It is preferred if the method is used for generating an immune response on a
15 human or other mammal by vaccinating said mammal.

It is preferred if the method is used for generating a therapeutic effect in a human or other mammal.

- 20 According to the present invention the above mentioned method is preferably used for the treatment of inflammatory disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic anemia,
25 macroglobulinaemia, trombocytopenic purpura, furthermore, for the management of bone disorders, cerebral oedema, Cogan's syndrome, congenital adrenal hyperplasia, connective tissue disorders, such as lichen, lupus erythematosus, polymyalgia rheumatica, polymyositis and dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves'
30 ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal vasculitis, scleritis, for some gastro-intestinal disorders, such as

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5 inflammatory bowel disease, nausea and oesophageal damage, for
hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis),
for Kawasaki disease, myasthenia gravis, various pain syndromes, such as
postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory
disorders, such as asthma, for the management of rheumatoid disease and
10 osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema,
erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis,
pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

Without any limitation of the scope of the present invention as defined by the attached
15 claims the invention shall now be described in more detail by referring to the following
examples and figures only showing non-limiting embodiments of the present invention.

General experimental set-up and sample preparation

20

Test formulation. Highly adaptable aggregate droplets used within the framework of
this work had the form of (oligo)bilayer vesicles. Typically, the test formulation
contained biocompatible (phospho)lipids, such as phosphatidylcholine, and
(bio)surfactants, such as sodium cholate or polysorbate (Tween 80). Different
25 phospholipid/detergent ratios have been chosen to maintain or select the highest
possible aggregate deformability.

Manufacturing was done as described in previous applications of the applicant. In
short, a solution of phosphatidylcholine (SPC; Natterman Phospholipids, Cologne,
30 Germany) in chloroform was labelled with the tritiated SPC (Amersham, XXX) and
mixed with sodium cholate (Merck, Darmstadt, Germany) to obtain a

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- 5 phospholipid/detergent ratio of 3.75/1 (mol/mol). The mixture was dispersed in phosphate buffer (pH = 7.2) to yield a 10 w-% total lipid suspension.

Vesicles in the suspension were frozen and thawed three times. Subsequently, the formulation was passed under pressure through several micro-porous filters (first 200
10 nm; then 100 nm, and finally 50 nm or 80 nm; Poretics, CA). To check the reproducibility of vesicle manufacturing, the average size of vesicles was measured with dynamic light scattering procedure and found to be in the range of 80 nm to 150 nm.

- 15 **Test animals.** Mice of NMRI strain were 8 to 12 weeks old at the time of experimentation. They had free access to standard chow and water and were kept in suspension cages in groups of 4 to 6. Prior to test formulation administration, the application area on each animals back was shaved carefully. The test preparation was administered under general anaesthesia (0.3 mL per mouse of an isotonic NaCl solution
20 containing 0.0071 % Rompun (Bayer, Leverkusen, Germany) and 14.3 mg/mL Ketavet (Parke-Davis, Rochester, N.Y). The administration was done with a high precision pipette on the skin which was left non-occluded. Each animal was finally transferred into an individual cage where it was kept for a day. A different cage was used for each animal for at least 24 hrs. 4 animals were used per test group.

- 25 **Test measurements.** Blood samples were collected from tail end, after termination of experiment at least. In one set of experiments, the early blood sampling was done every 2 hrs. Organ samples included: liver, spleen, kidney, and skin. The latter was also inspected superficially, by taking 10 strips (using a Tesa-Film).

- 30 Processing the organ samples was done according to standard procedures: for 3H-measurement, a small part of each organ and 100 µL of the carcass lysate were used to

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- 5 get the desired and quoted experimental data. These were analysed according to the standard procedures.

To determine total label recovery, the carcass of test animals was dissolved and discharged by addition of 50 mL perchloric acid

10

Recovery (% of applied activity) was determined and the recovered doses (% of applied activity per organ) as well as the total delivered amount [μg lipid/g organ] were calculated.

15 Examples 1-5:

Short term administration

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

20

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

trace amount of ^3H -DPPC with specific activity: $750 \mu\text{Ci}/500\mu\text{L}$

0.9 mL phosphate buffer, pH 7.3

Duration of experiment: 8 h .

- 25 Application area: 1 cm^2 on the upper dorsum. The various doses applied on the test area are given in the following table.

	Group 1	Group 2	Group 3	Group 4	Group 5
Applied volume [μL]	1.0	5.0	7.0	15.0	30.0
Appl. lipid amount [mg]	0.10	0.50	0.75	1.50	3.00
Applied activity [cpm]	108998	544991	817486	1634972	3269943

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5 Results of test measurements are given in figures 1 to 6.

Examples 6-8:

Longer term administration

10

Highly adaptable complex droplets (ultradeformable vesicles; Transfersomes)

87.4 mg phosphatidylcholine from soy bean (SPC)

12.6 mg sodium cholate (NaChol)

0.9 mL phosphate buffer, pH 7.3

15

trace amount of ^3H -DPPC with specific activity: 250 $\mu\text{Ci/mL}$

Duration of experiment: 24 h.

Application area: 1 cm squared; dose per area is given in the following table.

	Group 6	Group 7	Group 8
Applied volume [μL]	10.0	50.0	100.0
Appl. lipid amount [mg]	1.00	5.00	10.00
Applied activity [cpm]	145599	727997	1E+06

20 To test the effect of changing administered dose per area over longer period of time, even greater suspension volumes were applied on upper back of test mice.

Resulting data are analysed and presented together with those from previous experimental series in figures 1 to 7.

25

Figure 1 shows the recovery of relative activity (penetrant amount) in different layers of the skin as a function of applied activity (dose).

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- 5 Figure 2 shows the amount of carrier derived radioactivity (^3H -DPPC) in the blood as a function of time and epicutaneously administered penetrant quantity, expressed as percentage of applied dosage. As can be seen in this figure the relative amount of non-invasively administered lipid found in the blood reaches appreciable level after a clear lag-time of approximately 4 hours, but is nearly independent of the dose used.
- 10 Figure 3 indicates the relative accumulation of carrier derived radioactivity in various organs at two different time points after an increasing mass of ultradeformable carriers has been administered on the skin. It is apparent that whereas the relative amount of the carrier derived radioactivity decreases with the applied dosage at both times of exploration, the phospholipid amount in the blood, viable skin and liver in parallel
- 15 increases at $t = 8$ h, but remains nearly unchanged at $t = 24$ h.

Figure 4 shows the absolute penetrant distribution profile (in arbitrary units) in different layers of the skin as a function of applied activity (dose). Little dose dependence is seen in the horny layer for area doses between 0.5 mg cm^{-2} and up to 1.5

20 mg cm^{-2} , but greater penetrant amounts are deposited much more efficiently in the barrier. This is true 8 hours as well as 24 hours after the suspension administration. Viable skin accumulates the penetrant derived material in a dose dependent fashion in entire investigated range.

- 25 Figure 5 shows the total amount of penetrant recovered in different tissues (skin, blood, liver) at different times after the administration of an increasing quantity of ultradeformable penetrants on the skin grows with the applied dose per area. However, while at $t = 8$ h, an apparent saturation tendency is observed for doses greater than 1.5 mg cm^{-2} , at $t = 24$ h the dose dependence is linear.

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5 Figure 6 shows the time dependence of penetrant derived radioactivity in the blood as a function of epicutaneously administered suspension volume (lipid amount). As can be seen from this figure the temporal penetration characteristics are essentially independent of the applied dose: after a lag-time period of 4-6 hours, nearly steady state situation is observed.

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Figure 7 shows the penetrant derived radioactivity in the blood as a function of epicutaneously administered dose measured 8 h or 24 h after the application. Linear extrapolation suggests that barrier starts to adapt itself to penetrant transport at approximately 0.75 mg cm^{-2} .

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CLAIMS

1. A method for controlling the flux of penetrants across an adaptable semi-permeable porous barrier comprising the steps of:
- 10 – preparing a formulation by suspending or dispersing said penetrants in a polar liquid in the form of fluid droplets surrounded by a membrane-like coating of one or several layers, said coating comprising at least two kinds or forms of amphiphilic substances with a tendency to aggregate, provided that
 - 15 – said at least two substances differ by at least a factor of 10 in solubility in said polar liquid,
 - and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the
 - 20 diameter of homo-aggregates containing merely the less soluble substance,
 - and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating
 - 25 concentration in the unsolubilised droplet, whichever is higher;
 - and / or the presence of the more soluble substance lowers the average elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or
 - 30 of phospholipid bilayers with fluid aliphatic chains,

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- 5 – said penetrants being able to transport agents through the pores of said barrier or to enable agent permeation through the pores of said barrier after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants
- 10 across said barrier, and
- applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

15 2. The method according to claim 1,
characterised in that the flux across said barrier is increased by enlarging the applied dose per area of said penetrants.

 3. The method according to claims 1 or 2,
20 **characterised in that** the pH of the formulation is between 3 and 10, more preferably between 4 and 9, and most preferably between 5 and 8.

 4. The method according to any one of the preceding claims,
characterised in that the formulation comprises:

25 – at least one thickening agent in an amount that increases the formulation viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug retention at the application area is enabled,

 – and / or at least one antioxidant in an amount that reduces the increase of

30 oxidation index to less than 100 % per 6 months, more preferably to less

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- 5 than 100 % per 12 months and most preferably to less than 50 % per
12 months
- and / or at least one microbicide in an amount that reduces the bacterial
count of 1 million germs added per g of total mass of the formulation to
less than 100 in the case of aerobic bacteria, to less than 10 in the case of
10 entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*
or *Staphilococcus aureus*, after a period of 4 days.

5. The method according to claim 4,
characterised in that said at least one microbicide is added in an amount
15 that reduces the bacterial count of 1 million germs added per g of total mass
of the formulation to less than 100 in the case of aerobic bacteria, to less
than 10 in the case of entero-bacteria, and to less than 1 in the case of
Pseudomonas aeruginosa or *Staphilococcus aureus*, after a period of 3 days,
and more preferably after a period of 1 day.

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6. The method according to claim 4,
characterised in that said thickening agent is selected from the class of
pharmaceutically acceptable hydrophilic polymers, such as partially
etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-,
25 hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely
synthetic hydrophilic polymers such as polyacrylates , polymethacrylates,
poly(hydroxyethyl)-, poly(hydroxypropyl)-,
poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-
sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,
30 polyethylene glycol-lactides, polyethylene glycol-diacrylates,
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),

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5 poly(propylene fumarate-co-ethylene glycols), poloxamers,
polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;
natural gums comprising alginates, carrageenans, guar-gums, gelatines,
tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;
mixtures and further derivatives or co-polymers thereof and / or other
10 pharmaceutically, or at least biologically, acceptable polymers.

7. The method according to claim 6,
characterised in that the concentration of said polymer is in the range
between 0.01 w- % and 10 w- %, more preferably in the range between 0.1
15 w- % and 5 w- %, even more preferably in the range between 0.25 w- % and
3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

8. The method according to claim 4,
characterised in that said anti-oxidant is selected from synthetic phenolic
20 antioxidants, such as butylated hydroxyanisol (BHA), butylated
hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548,
HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.),
tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-
trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine,
25 p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol,
tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol,
hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid,
quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic
acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta,
30 gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.
-acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other

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- 5 suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and corresponding amide- and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g. 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.); non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin,
- 10 diclofenac, mefenamic acid, flufenamic acid, phenylbutazone, oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen, ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine, quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital, acetaminophen); aminosalicylic acids and derivatives; methotrexate,
- 15 probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol), ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral;
- 20 endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids
- 25 (e.g. flavones, flavonols, flavonones, flavanones, chalcones, anthocyanins), N-acetylcystein, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, hydroxycinnamic acids and their esters (e.g. coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon,
- 30 sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid;

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5 oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

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9. The method according to claim 8,
characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between
15 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably
20 is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001
25 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cystein is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between
30 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between

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5 0.001–0.2 w-%, and most preferably is between 0.005–0.02 w-%, most typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most
10 preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01–0.2, most typically between 0.3 and 2 w-%.

15 10. The method according claim 4,
characterised in that said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xilenol,
20 dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts; quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts,
25 e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

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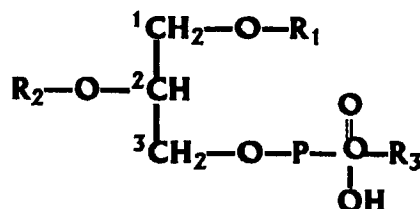
5 11. The method according to claim 10,
 characterised in that the bulk concentration of short chain alcohols in the
 case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more
 preferably is up to 5 w-%, and most preferably is in the range between
 0.5-3 w-%, and in the case of chlorobutanol is in the range between
10 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of
 methyl paraben is in the range between 0.05-0.2 w-%, and in the case of
 propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration
 of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of
 benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of
15 phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk
 concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

 12. The method according to any one of the preceding claims,
 characterised in that the less soluble amongst the aggregating substances is
20 a lipid or lipid-like material, especially a polar lipid, whereas the substance
 which is more soluble in the suspending liquid and which lowers the
 average elastic energy of the droplet is a surfactant or else has surfactant-
 like properties and / or is a form of said lipid or lipid-like material which is
 comparably soluble as said surfactant or the surfactant-like material.

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 13. Formulation according to claim 12,
 characterised in that the lipid or lipid-like material is a lipid or a lipid
 from a biological source or a corresponding synthetic lipid or any of its
 modifications, said lipid preferably belonging to the class of pure
30 phospholipids corresponding to the general formula

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where R_1 and R_2 is an aliphatic chain, typically a C_{10-20} -acyl, or -alkyl or partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-,
 10 myristoyl-, palmitoyl-, or stearoyl chain; and where R_3 is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C_{1-4} -alkyl, C_{1-5} -alkyl substituted with carboxy, C_{2-5} -alkyl substituted with hydroxy, C_{2-5} -alkyl substituted with carboxy and hydroxy, or C_{2-5} -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also
 15 glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids,
 20 phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-
 25 groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

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- 5 14. Formulation according to claim 12,
characterised in that the surfactant or surfactant-like material is a nonionic,
a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -
alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a
monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate,
10 taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-
aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-
methylglucamide, N- alkyl-N,N- dimethylglycine, 3-
(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a
polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-
15 octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl
ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-
isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether,
polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-
monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate
20 (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-
lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-
4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the
corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -
laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-
25 monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl-
or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-
methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate,
sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium
taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium
30 laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-
glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-

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5 acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol,
or -phosphorylserine, n-tetradecyl- glycero-phosphatidic acid, -
phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-,
elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain
phospholipid, or else a surface-active polypeptide.

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15. The method according to any of the preceding claims,
characterised in that the average diameter of the penetrant is between
30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more
preferably between 50 nm and 200 nm and particularly preferably between
15 60 nm and 150 nm.

16. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is
0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably
20 between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and
20 w-%.

17. The method according to any one of the preceding claims,
characterised in that the total dry weight of droplets in a formulation is
25 selected to increase the formulation viscosity to maximally 5 Nm/s, more
preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that
formulation spreading-over and drug retention at the application area is
enabled.

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5 18. The method according to any one of the preceding claims,
 characterised in that at least one edge-active substance or surfactant and/or
 at least one amphiphilic substance, and / or at least one hydrophilic fluid and
 the agent are mixed, if required separately, to form a solution, the resulting
 (partial) mixtures or solutions are then combined subsequently to induce,
10 preferably by action of mechanical energy such as shaking, stirring,
 vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,
 or filtration using convenient driving pressure, the formation of penetrants
 that associate with and / or incorporate the agent

15 19. The method of claim 18,
 characterised in that said amphiphilic substances are dissolved in volatile
 solvents, such as alcohols, especially ethanol, or in other pharmaceutically
 acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl
20 alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400
 D) or glycerol, other pharmaceutically acceptable organic solvents, such as
 undercooled gas, especially supercritical CO₂, which are then removed,
 especially by evaporation or dilution, prior to making the final preparation.

25 20. The method according to any one of claims 18 or 19,
 characterised in that the formation of said penetrants is induced by the
 addition of required substances into a fluid phase, evaporation from a
 reverse phase, by injection or dialysis, if necessary under the influence of
30 mechanical stress, such as shaking, stirring, in especially high velocity
 stirring, vibrating, homogenising, ultrasonication, shearing, freezing and

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5 thawing, or filtration using convenient, in especially low (1 MPa) or
intermediate (up to 10 MPa), driving pressure.

21. The method of claim 20,
characterised in that the formation of said penetrants is induced by
10 filtration, the filtering material having pores sizes between 0.01 μm and
0.8 μm , more preferably between 0.02 μm and 0.3 μm , and most preferably
between 0.05 μm and 0.15 μm , whereby several filters may be used
sequentially or in parallel.

15 22. The method according to any one of claims 18 to 21,
characterised in that said agents and penetrants are made to associate, at
least partly,
– after the formation of said penetrants, e.g. after injecting a solution of the
drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and
20 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol
(molecular weight: 200-400 D) or glycerol into the suspending medium,
– simultaneously with penetrant formation, if required using the drug
co-solution and, at least some, penetrant ingredients.

25 23. The method according to any one of the claims 18 to 22,
characterised in that said penetrants, with which the agent is associated,
are prepared immediately before the application of the formulation, if
convenient, from a suitable concentrate or a lyophilisate.

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5 24. The method according to any one of the preceding claims,
characterised in that the formulation is applied by spraying, smearing,
rolling or sponging on the application area, in particular by using a metered
sprayer, spender, roller, sponge or a non-occlusive patch, as appropriate.

10 25. The method according to any one of the preceding claims,
characterised in that the barrier is a part of a mammalian body and / or a
plant and preferably is skin and / or at least partly keratinised endothelium
and / or nasal or any other mucosa.

15 26. The method according to claim 25,
characterised in that, the area dose of said penetrant is between 0.1 mg per
square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between
 0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between
20 0.5 mg cm^{-2} and 15 mg cm^{-2} , in case the penetrant is applied on said skin
and / or said at least partly keratinised endothelium.

 27. The method according to claim 25,
characterised in that the area dose of said penetrant is between 0.05 mg per
25 square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between
 0.1 mg cm^{-2} and 15 mg cm^{-2}) and even more preferably is between
 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case the penetrant is applied on said
nasal or other mucosa.

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5 28. The method according to claim 25,
 characterised in that the area dose of said penetrant is between 0.0001 mg
 per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is
 between $0.0005 \text{ mg cm}^{-2}$ and 0.05 mg cm^{-2} and even more preferably is
 between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is
10 applied on plant body, plant leaves or plant needles.

 29. A kit containing said formulation in an amount which enables
 the formulation to be applied at the selected dose per area, according to any
 one of the preceding claims.
15

 30. The kit according to claim 29,
 characterised in that the formulation is contained in a bottle or any other
 packaging vessel.

20 31. The kit according to claims 29 or 30,
 characterised in that it contains a device for administering the formulation.

 32. The kit according to claim 31,
 characterised in that the device is a non-occlusive patch.

25 33. A device comprising a non-occlusive patch, containing the
 formulation as in any one of claims 1 to 27 in an amount that yields the dose
 per area according to any one of the preceding claims.

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- 5 34. A non-occlusive patch according to claim 33,
 comprising a laminated composite of:
- a backing layer;
 - an active agent-permeable membrane, the backing layer and membrane
 defining
 - 10 — a reservoir therebetween that contains the formulation of the active agent,
 said reservoir having a smaller periphery than the backing layer and
 membrane such that a portion of the backing layer and membrane extends
 outwardly of the periphery of the reservoir;
 - a pressure sensitive adhesive layer that underlies and covers the active
 - 15 agent-permeable membrane and said outwardly extending portion of the
 backing layer and membrane.

35. A non-occlusive patch according to claim 33,
 comprising a laminated composite of:
- 20 — a backing layer;
 - a matrix layer that contains the formulation of the active agent; and
 - a pressure sensitive adhesive layer.

36. The device according to claim 33,
- 25 **characterised in that** the formulation and / or agent and / or
 suspension / dispersion of penetrants without the agent are kept during the
 storage in several, more preferably less than 5, even more preferably in 3,
 and most preferred in less than 3 separate inner compartments of the device
 which, in case, are combined prior to or during the application of the
- 30 formulation.

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5 37. The device according to claim 36,
 characterised in that said compartment(s) filled with the formulation
 and / or agent and / or suspension of penetrants without the agent, is (are)
 covered, on one or both sides, with a non-occlusive, semi-permeable
 membrane that lets small molecules, such as water, but only few or not the
10 penetrants pass.

 38. The device according to claim 37,
 characterised in that said non-occlusive, semi-permeable membrane is the
15 same or different, if it is used on both sides of said device.

 39. The device according to claims 37 or 38,
 characterised in that the water permeability of said semi-permeable but
 non-occlusive membrane is at least $10 \text{ mg cm}^{-2} \text{ h}^{-1}$, more preferably exceeds
20 $50 \text{ mg cm}^{-2} \text{ h}^{-1}$ and most preferably is greater than $100 \text{ mg cm}^{-2} \text{ h}^{-1}$.

 40. The device according to claims 37 to 39,
 characterised in that the area of said semi-permeable membrane is between
 0.5 cm^2 and 250 cm^2 , more preferably is between 1 cm^2 and 100 cm^2 , even
25 more preferably is between 2 cm^2 and 50 cm^2 and most preferred is between
 4 cm^2 and 25 cm^2 .

 41. The device according to claims 37 to 40,
 characterised in that the area of said semi-permeable membrane is the area
30 substantially covered by the formulation filled part of the device.

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- 5 42. The device according to claims 37 to 41,
characterised in that the penetrant flux across the barrier is controlled by
the permeability of, or the suspension-medium evaporation across, the
semi-permeable, non-occlusive membrane
- 10 43. The device according to claims 37 to 42,
characterised in that the device is filled with the formulation and / or agent
molecules and / or suspension of penetrants without agent, either separately
or together, prior to the administration of said patch, preferably 360 min,
more preferably 60 min, even more preferably 30 min and most
15 preferably within few minutes before placing the device on the barrier.
44. A method of administering an agent onto a mammalian body
or a plant, for transporting said agent through a barrier, such as the intact
20 skin/mucosa or cuticle, respectively, when the agent is associated with the
penetrant which is capable of transporting said agent through the skin pores
or through the passages in mucosa or cuticle, or else is capable of enabling
agent permeation through skin pores after said penetrant has opened and/or
entered said pores, comprising the steps of:
- 25 – preparing a formulation by suspending or dispersing said penetrants in a
polar liquid in the form of fluid droplets surrounded by a membrane-like
coating of one or several layers, said coating comprising at least two
kinds or forms of amphiphilic substances with a tendency to aggregate,
provided that
- 30 – said at least two substances differ by at least a factor of 10 in solubility in
said polar liquid,

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- 5 – and / or said substances when in the form of homo-aggregates (for the more soluble substance) or of hetero-aggregates (for any combination of both said substances) have a preferred average diameter smaller than the diameter of homo-aggregates containing merely the less soluble substance,
- 10 – and / or the more soluble substance tends to solubilise the droplet and the content of such substance is to up to 99 mol-% of solubilising concentration or else corresponds to up to 99 mol-% of the saturating concentration in the unsolubilised droplet, whichever is higher,
- and / or the presence of the more soluble substance lowers the average
15 elastic energy of the membrane-like coating to a value at least 5 times lower, more preferably at least 10 times lower and most preferably more than 10 times lower, than the average elastic energy of red blood cells or of phospholipid bilayers with fluid aliphatic chains,
- said penetrants being able to transport agents through the pores of said
20 barrier or being able to promote agent permeation through the pores of said skin after penetrants have entered the pores,
- selecting a dose amount of said penetrants to be applied on a predetermined area of said barrier to control the flux of said penetrants across said barrier, and
- 25 – applying the selected dose amount of said formulation containing said penetrants onto said area of said porous barrier.

45. The method according to claim 44,
characterised in that the flux of penetrants across said barrier is increased
30 by enlarging the applied dose per area of said penetrants.

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5 46. The method according to claims 44 or 45,
 characterised in that the pH of the formulation is between 3 and 10, more
 preferably between 4 and 9, and most preferably between 5 and 8.

 47. The method according to claims 44 to 46,
10 **characterised in that** the formulation comprises:
 – at least one thickening agent in an amount that increases the formulation
 viscosity to maximally 5 Nm/s, more preferably up to 1 Nm/s, and most
 preferably up to 0.2 Nm/s, so that formulation spreading-over, and drug
 retention at the application area is enabled,
15 – and / or at least one antioxidant in an amount that reduces the increase of
 oxidation index to less than 100 % per 6 months, more preferably to less
 than 100 % per 12 months and most preferably to less than 50 % per
 12 months
 – and / or at least one microbicide in an amount that reduces the bacterial
20 count of 1 million germs added per g of total mass of the formulation to
 less than 100 in the case of aerobic bacteria, to less than 10 in the case of
 entero-bacteria, and to less than 1 in the case of *Pseudomonas aeruginosa*
 or *Staphylococcus aureus*, after a period of 4 days.

25 48. Formulation according to claim 47,
 characterised in that said at least one microbicide is added in an amount
 that reduces the bacterial count of 1 million germs added per g of total mass
 of the formulation to less than 100 in the case of aerobic bacteria, to less
30 than 10 in the case of entero-bacteria, and to less than 1 in the case of

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- 5 *Pseudomonas aeruginosa* or *Staphylococcus aureus*, after a period of 3 days,
and more preferably after a period of 1 day.

49. The method according to claim 47,
characterised in that said thickening agent is selected from the class of
10 pharmaceutically acceptable hydrophilic polymers, such as partially
etherified cellulose derivatives, like carboxymethyl-, hydroxyethyl-,
hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely
synthetic hydrophilic polymers such as polyacrylates, polymethacrylates,
poly(hydroxyethyl)-, poly(hydroxypropyl)-,
15 poly(hydroxypropylmethyl)methacrylates, polyacrylonitriles, methallyl-
sulphonates, polyethylenes, polyoxiethylenes, polyethylene glycols,
polyethylene glycol-lactides, polyethylene glycol-diacrylates,
polyvinylpyrrolidones, polyvinyl alcohols, poly(propylmethacrylamides),
poly(propylene fumarate-co-ethylene glycols), poloxamers,
20 polyaspartamides, (hydrazine cross-linked) hyaluronic acids, silicones;
natural gums comprising alginates, carrageenans, guar-gums, gelatines,
tragacanth, (amidated) pectins, xanthans, chitosan collagens, agaroses;
mixtures and further derivatives or co-polymers thereof and / or other
pharmaceutically, or at least biologically, acceptable polymers.

25

50. The method according to claim 49,
characterised in that the concentration of said polymer is in the range
between 0.01 w- % and 10 w- %, more preferably in the range between 0.1
w- % and 5 w- %, even more preferably in the range between 0.25 w- % and
30 3.5 w- % and most preferably in the range between 0.5 w- % and 2 w- %.

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- 5 51. The method according to claim 47,
 characterised in that said anti-oxidant is selected from synthetic phenolic
 antioxidants, such as butylated hydroxyanisol (BHA), butylated
 hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548,
 HWA-131, BF-389, CI-986, PD-127443, E-5119, BI-L-239XX, etc.),
10 tertiary butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-
 trimethylhydroquinone (HTHQ); aromatic amines (such as diphenylamine,
 p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol,
 tetrahydroindenoindol); phenols and phenolic acids (such as guaiacol,
 hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid,
15 quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic
 acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta,
 gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g.
 -acetate, -laurate, myristate, -palmitate, -oleate, -linoleate, etc., or any other
 suitable tocopheryl-lipoate), tocopheryl-POE-succinate; trolox and
20 corresponding amide- and thiocarboxamide analogues; ascorbic acid and its
 salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g.
 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.);
 non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin,
 diclofenac, mefenamic acid, flufenamic acid, phenylbutazone,
25 oxyphenbutazone acetylsalicylic acid, naproxen, diflunisal, ibuprofen,
 ketoprofen, piroxicam, penicillamine, penicillamine disulphide, primaquine,
 quinacrine, chloroquine, hydroxychloroquine, azathioprine, phenobarbital,
 acetaminophen); aminosalicylic acids and derivatives; methotrexate,
 probucol, antiarrhythmics (e.g. amiodarone, aprindine, asocainol),
30 ambroxol, tamoxifen, b-hydroxytamoxifen; calcium antagonists (such as
 nifedipine, nisoldipine, nimodipine, nicardipine, nilvadipine), beta-receptor

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5 blockers (e.g. atenolol, propranolol, nebivolol); sodium bisulphite, sodium metabisulphite, thiourea; chelating agents, such as EDTA, GDTA, desferral; endogenous defence systems, such as transferrin, lactoferrin, ferritin, ceruloplasmin, haptoglobin, haemopexin, albumin, glucose, ubiquinol-10; enzymatic antioxidants, such as superoxide dismutase and metal complexes
 10 with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta-carotene, bilirubin, uric acid; flavonoids (e.g. flavones, flavonols, flavonones, flavanonals, chalcones, anthocyanins), N-acetylcysteine, mesna, glutathione, thiohistidine derivatives, triazoles; tannins, cinnamic acid, hydroxycinnamic acids and their esters (e.g.
 15 coumaric acids and esters, caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g. from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsoic acid; rosmarinic acid, rosmarindiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers,
 20 sulphoxides, tetraalkylthiuram disulphides; phytic acid, steroid derivatives (e.g. U74006F); tryptophan metabolites (e.g. 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides, or else is an oxidation suppressing enzyme.

25 52. The method according to claim 51,
characterised in that the concentration of BHA or BHT is between 0.001 and 2 w-%, more preferably is between 0.0025 and 0.2 w-%, and most preferably is between 0.005 and 0.02 w-%, of TBHQ and PG is between 0.001 and 2 w-%, more preferably is between 0.005 and 0.2 w-%, and most
 30 preferably is between 0.01 and 0.02 w-%, of tocopherols is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most

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5 preferably is between 0.05 and 0.075 w-%, of ascorbic acid esters is between 0.001 and 5, more preferably is between 0.005 and 0.5, and most preferably is between 0.01 and 0.15 w-%, of ascorbic acid is between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.1 w-%, of sodium bisulphite or sodium metabisulphite is
10 between 0.001 and 5, more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01-0.15 w-%, of thiourea is between 0.0001 and 2 w-%, more preferably is between 0.0005 and 0.2, and most preferably is between 0.001-0.01 w-%, most typically 0.005 w-%, of cysteine is between 0.01 and 5, more preferably is between 0.05 and 2 w-%, and most
15 preferably is between 0.1 and 1.0 w-%, most typically 0.5 w-%, of monothioglycerol is between 0.01 and 5 w-%, more preferably is between 0.05 and 2 w-%, and most preferably is between 0.1-1.0 w-%, most typically 0.5 w-%, of NDGA is between 0.0005-2 w-%, more preferably is between 0.001-0.2 w-%, and most preferably is between 0.005-0.02 w-%, most
20 typically 0.01 w-%, of glutathione is between 0.005 and 5 w-%, more preferably is between 0.01 and 0.5 w-%, and most preferably is between 0.05 and 0.2 w-%, most typically 0.1 w-%, of EDTA is between 0.001 and 5 w-%, even more preferably is between 0.005 and 0.5 w-%, and most preferably is between 0.01 and 0.2 w-%, most typically between 0.05 and
25 0.975 w-%, of citric acid is between 0.001 and 5 w-%, even more preferably is between 0.005 and 3 w-%, and most preferably is between 0.01-0.2, most typically between 0.3 and 2 w-%.

53. The method according claim 47,
30 **characterised in that** said microbicide is selected amongst short chain alcohols, such as ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol,

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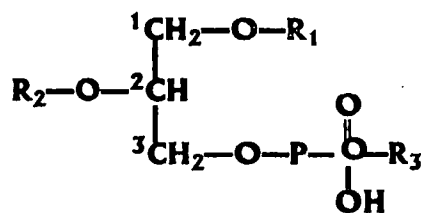
- 5 chlorbenzyl alcohol, dichlorbenzylalcohol; hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xlenol, dichlorophene, hexachlorophene, povidon-iodine; parabens, especially alkyl-paraben, such as methyl-, ethyl-, propyl-, or butyl-paraben, benzyl-paraben; acids, such as sorbic acid, benzoic acid and its salts;
- 10 quaternary ammonium compounds, such as alkonium salts, e.g. benzalkonium salts, especially the chlorides or bromides, cetrimonium salts, e.g. the bromide; phenoalkecinium salt, such as phenododecinium bromide, cetylpyridinium chloride or other such salts; mercurium compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal; chlorhexidine or its
- 15 gluconate; antibiotically active compounds of biological origin, or a mixture thereof.

54. The method according claim 53,
- characterised in that** the bulk concentration of short chain alcohols in the
- 20 case of ethyl, propyl, butyl or benzyl alcohol is up to 10 w-%, more preferably is up to 5 w-%, and most preferably is in the range between 0.5-3 w-%, and in the case of chlorobutanol is in the range between 0.3-0.6 w-%; bulk concentration of parabens, especially in the case of methyl paraben is in the range between 0.05-0.2 w-%, and in the case of
- 25 propyl paraben is in the range between 0.002-0.02 w-%; bulk concentration of sorbic acid is in the range between 0.05-0.2 w-%, and in the case of benzoic acid is in the range between 0.1-0.5 w-%; bulk concentration of phenols, triclosan, is in the range between 0.1-0.3 w-%, and bulk concentration of chlorhexidine is in the range between 0.01-0.05 w-%.

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5 55. The method according to claims 44 to 54,
 characterised in that the less soluble amongst the aggregating substances is
 a lipid or lipid-like material, especially a polar lipid, whereas the substance
 which is more soluble in the suspending liquid and which lowers the
 average elastic energy of the droplet is a surfactant or else has surfactant-
 10 like properties and / or is a form of said lipid or lipid-like material which is
 comparably soluble as said surfactant or the surfactant-like material.

 56. Formulation according to claim 55,
 characterised in that the lipid or lipid-like material is a lipid or a lipoid
 15 from a biological source or a corresponding synthetic lipid or any of its
 modifications, said lipid preferably belonging to the class of pure
 phospholipids corresponding to the general formula



20 where R₁ and R₂ is an aliphatic chain, typically a C₁₀₋₂₀-acyl, or -alkyl or
 partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoeloyl-,
 elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vaccinyl-, lauroyl-,
 myristoyl-, palmitoyl-, or stearoyl chain; and where R₃ is hydrogen,
 25 2-trimethylamino-1-ethyl, 2-amino-1-ethyl, C₁₋₄-alkyl, C₁₋₅-alkyl substituted
 with carboxy, C₂₋₅-alkyl substituted with hydroxy, C₂₋₅-alkyl substituted with

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5 carboxy and hydroxy, or C₂₋₅-alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer-forming lipids, in particular half-protonated fluid fatty acids, said lipid is selected from the
10 group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebrosides, ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides and other glycolipids or
15 synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be ester-groups-linked to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids.

20 57. Formulation according to claim 55,
characterised in that the surfactant or surfactant-like material preferably is a nonionic, a zwitterionic, an anionic or a cationic surfactant, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate,
25 glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl- aminoxide, esp. a dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N- alkyl-N,N- dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-
30 octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-

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5 isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-lauryl, -myristoyl, -cetylstearyl, or -oleoyl ether, as in polyhydroxyethylene-
 10 4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), -laurate or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-
 15 methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl- or oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, such as sodium elaidate, sodium linoleate, sodium laurate, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-
 20 acyl-, e.g. lauryl or oleoyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-tetradecyl- glycero-phosphatidic acid, -phosphorylglycerol, or - phosphorylserine, a corresponding palmitoeloyl-, elaidoyl-, vaccenyl-lysophospholipid or a corresponding short-chain phospholipid, or else a surface-active polypeptide.

25

58. The method according to claims 44 to 57,

characterised in that the average diameter of the penetrant is between 30 nm and 500 nm, more preferably between 40 nm and 250 nm, even more preferably between 50 nm and 200 nm and particularly preferably between
 30 60 nm and 150 nm.

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5 59. The method according to claims 44 to 58,
 characterised in that the total dry weight of droplets in a formulation is
 0.01 weight-% (w-%) to 40 w-% of total formulation mass, more preferably
 between 0.1 w-% and 30 w-%, and most preferably between 0,5 w-% and
 20 w-%.

10

 60. The method according to claims 44 to 59,
 characterised in that the total dry weight of droplets in a formulation is
 selected to increase the formulation viscosity to maximally 5 Nm/s, more
 preferably up to 1 Nm/s, and most preferably up to 0.2 Nm/s, so that
15 formulation spreading-over and drug retention at the application area is
 enabled.

 61. The method according to claims 44 to 60,
 characterised in that at least one edge-active substance or surfactant and/or
20 at least one amphiphilic substance, and / or at least one hydrophilic fluid and
 the agent are mixed, if required separately, to form a solution, the resulting
 (partial) mixtures or solutions are then combined subsequently to induce,
 preferably by action of mechanical energy such as shaking, stirring,
 vibrations, homogenisation, ultrasonication, shearing, freezing and thawing,
25 or filtration using convenient driving pressure, the formation of penetrants
 that associate with and / or incorporate the agent

 62. The method according to claim 61,
 characterised in that said amphiphilic substances are dissolved in volatile
30 solvents, such as alcohols, especially ethanol, or in other pharmaceutically
 acceptable organic solvents, such as ethanol, 1- and 2-propanol, benzyl

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5 alcohol, propylene glycol, polyethylene glycol (molecular weight: 200-400 D) or glycerol, other pharmaceutically acceptable organic solvents, such as undercooled gas, especially supercritical CO₂, which are then removed, especially by evaporation or dilution, prior to making the final preparation.

10 63. The method according to any one of claims 61 or 62,
characterised in that the formation of said penetrants is induced by the addition of required substances into a fluid phase, evaporation from a reverse phase, by injection or dialysis, if necessary under the influence of mechanical stress, such as shaking, stirring, especially high velocity stirring,
15 vibrating, homogenising, ultrasonication, shearing, freezing and thawing, or filtration using a convenient, especially low (1 MPa) or intermediate (up to 10 MPa), driving pressure.

20 64. The method according to claim 63,
characterised in that the formation of said penetrants is induced by filtration, the filtering material having pores sizes between 0.01 µm and 0.8 µm, more preferably between 0.02 µm and 0.3 µm, and most preferably between 0.05 µm and 0.15 µm, whereby several filters may be used
25 sequentially or in parallel.

 65. The method according to any one of claims 45 to 64,
characterised in that said agents and penetrants are made to associate, at least partly,
30 – after the formation of said penetrants, e.g. after injecting a solution of the drug in a pharmaceutically acceptable fluid, such as ethanol, 1- and

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- 5 2-propanol, benzyl alcohol, propylene glycol, polyethylene glycol
(molecular weight: 200-400 D) or glycerol into the suspending medium,
– simultaneously with penetrant formation, if required using the drug
co-solution and, at least some, penetrant ingredients.

10 66. The method according to any one of the claims 45 to 65,
characterised in that said penetrants, with which the agent is associated,
are prepared immediately before the application of the formulation, if
convenient, from a suitable concentrate or a lyophilisate.

15 67. The method according to any one of the claims 45 to 66,
characterised in that the formulation is applied by spraying, smearing,
rolling or sponging on the application area, in particular by using a metered
sprayer, spender, roller or a sponge, or a non-occlusive patch, as appropriate.

20 68. The method according to any one of the claims 45 to 67,
characterised in that the barrier is skin or at least partly keratinised
endothelium and / or nasal or any other mucosa.

 69. The method according to claim 68,
25 **characterised in that**, the area dose of said penetrant is between 0.1 mg per
square centimetre (mg cm^{-2}) and 40 mg cm^{-2} , more preferably is between
0.25 mg cm^{-2} and 30 mg cm^{-2} and even more preferably is between
0.5 mg cm^{-2} and 15 mg cm^{-2} , in the case that the penetrant is applied on
said skin and / or said at least partly keratinised endothelium.

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5 70. The method according to claim 68,
 characterised in that the area dose of said penetrant is between 0.05 mg per
 square centimetre (mg cm^{-2}) and 20 mg cm^{-2} , more preferably is between
 0.1 mg cm^{-2} and 15 mg cm^{-2} and even more preferably is between
 0.5 mg cm^{-2} and 10 mg cm^{-2} , in the case that the penetrant is applied on
10 said nasal or other mucosa.

 71. The method according to claim 68,
 characterised in that the area dose of said penetrant is between 0.0001 mg
 per square centimetre (mg cm^{-2}) and 0.1 mg cm^{-2} , more preferably is
15 between 0.0005 mg cm^{-2} and 0.05 mg cm^{-2} and even more preferably is
 between 0.001 mg cm^{-2} and 0.01 mg cm^{-2} , in the case that the penetrant is
 applied on plant body, plant leaves or plant needles.

 72. The method of claim 44, used for generating an immune
20 response on a human or other mammal by vaccinating said mammal.

 73. The method of claim 44, used for generating a therapeutic
 effect in a human or other mammal.

25 74. The method of claim 44 for the treatment of inflammatory
 disease, dermatosis, kidney or liver failure, adrenal insufficiency, aspiration
 syndrome, Behcet syndrome, bites and stings, blood disorders, such as cold-
 haemagglutinin disease, haemolytic anemia, hypereosinophilia, hypoplastic
 anemia, macroglobulinaemia, thrombocytopenic purpura, furthermore, for the
30 management of bone disorders, cerebral oedema, Cogan's syndrome,
 congenital adrenal hyperplasia, connective tissue disorders, such as lichen,

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- 5 lupus erythematosus, polymyalgia rheumatica, polymyositis and
dermatomyositis, epilepsy, eye disorders, such as cataracts, Graves'
ophthalmopathy, haemangioma, herpes infections, neuropathies, retinal
vasculitis, scleritis, for some gastro-intestinal disorders, such as
inflammatory bowel disease, nausea and oesophageal damage, for
10 hypercalcaemia, infections, e.g. of the eye (as in infections mononucleosis),
for Kawasaki disease, myasthenia gravis, various pain syndromes, such as
postherpetic neuralgia, for polyneuropathies, pancreatitis, in respiratory
disorders, such as asthma, for the management of rheumatoid disease and
osteoarthritis, rhinitis, sarcoidosis, skin diseases, such as alopecia, eczema,
15 erythema multiforme, lichen, pemphigus and pemphigoid, psoriasis,
pyoderma gangrenosum, urticaria, in case of thyroid and vascular disorders.

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Figure 1

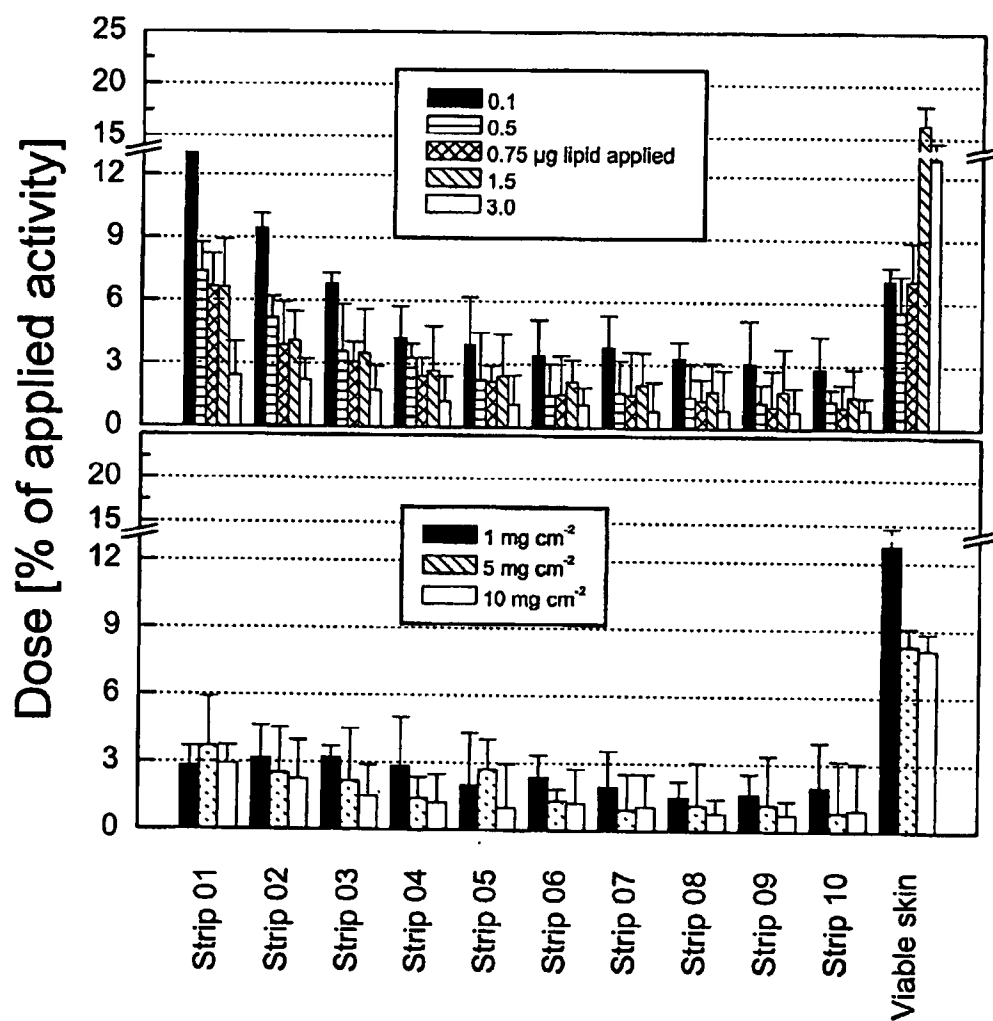


Figure 2

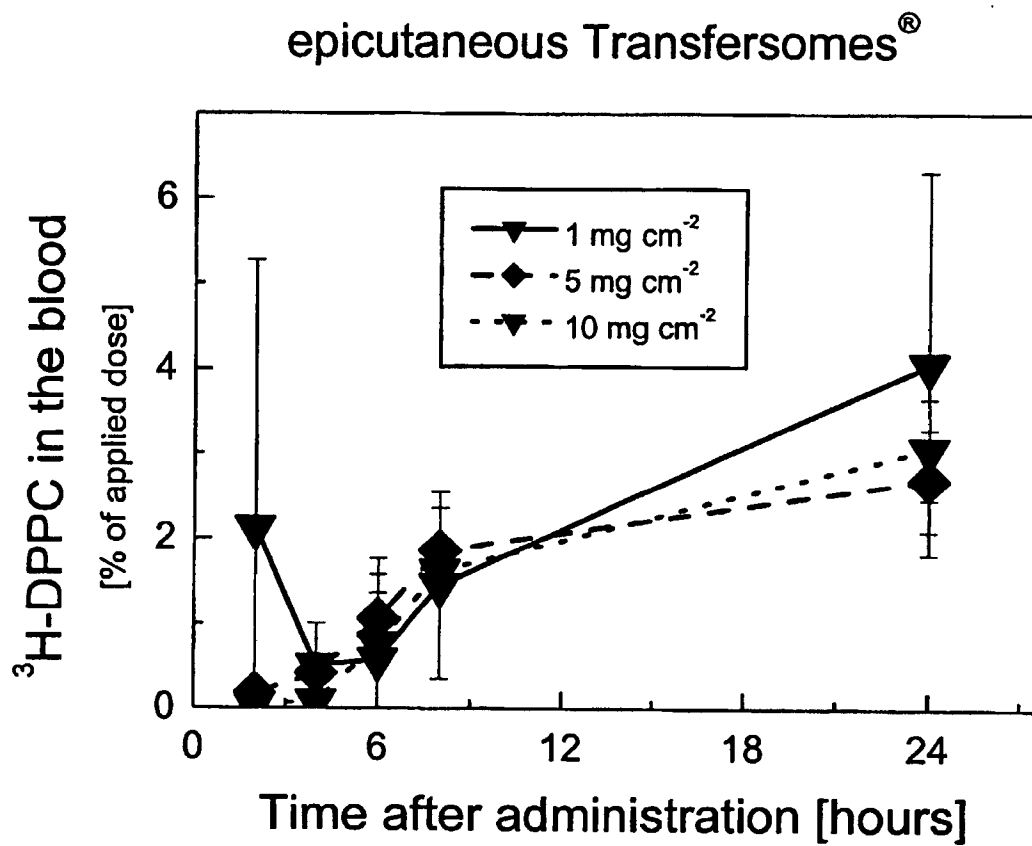


Figure 3

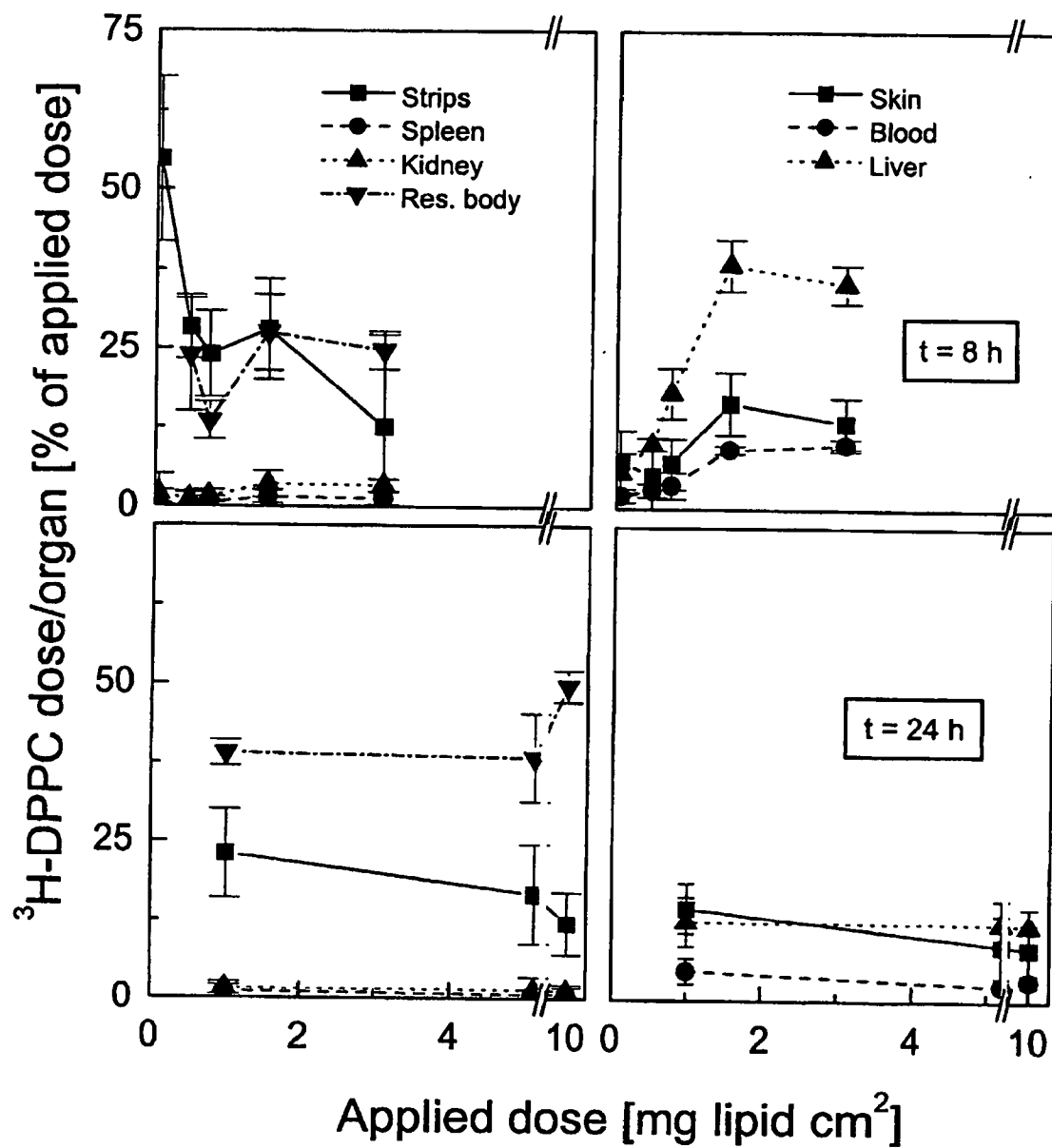


Figure 4

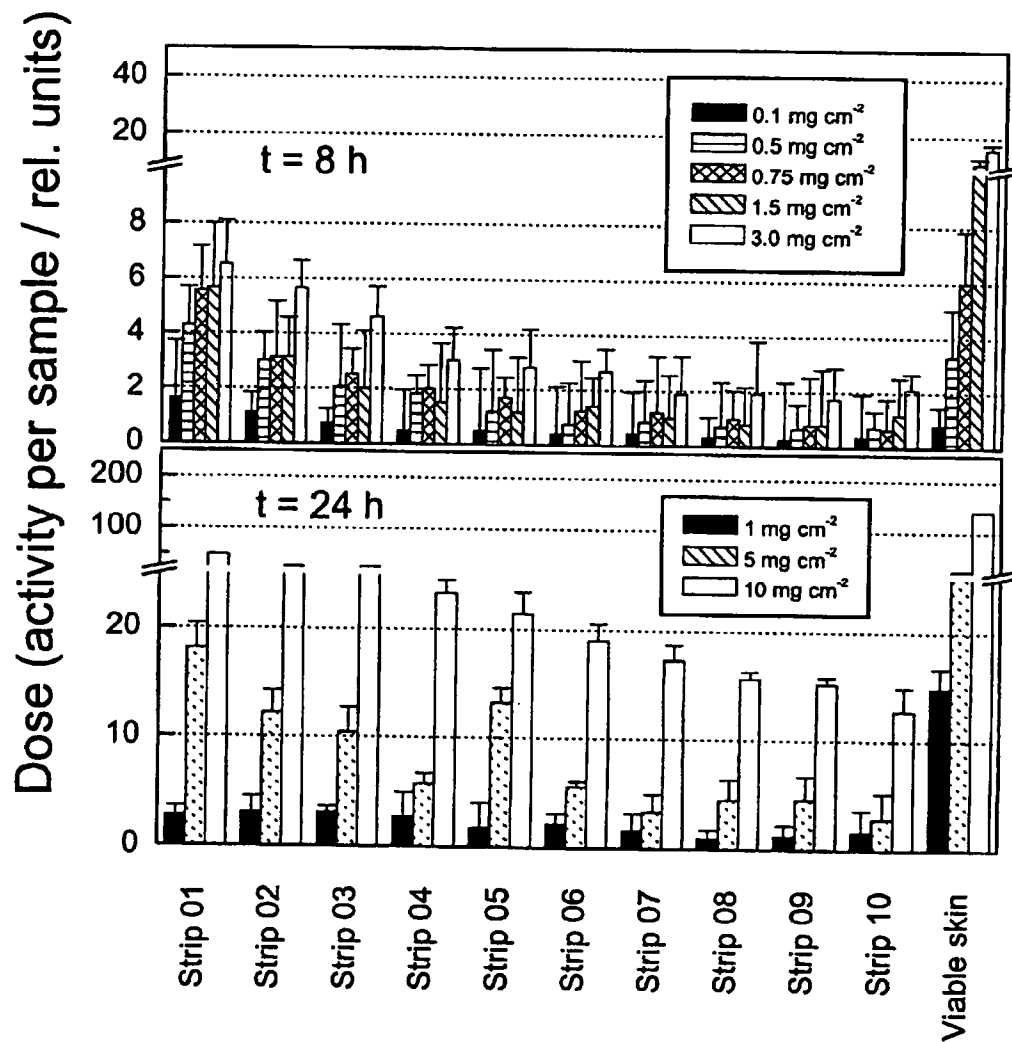


Figure 5

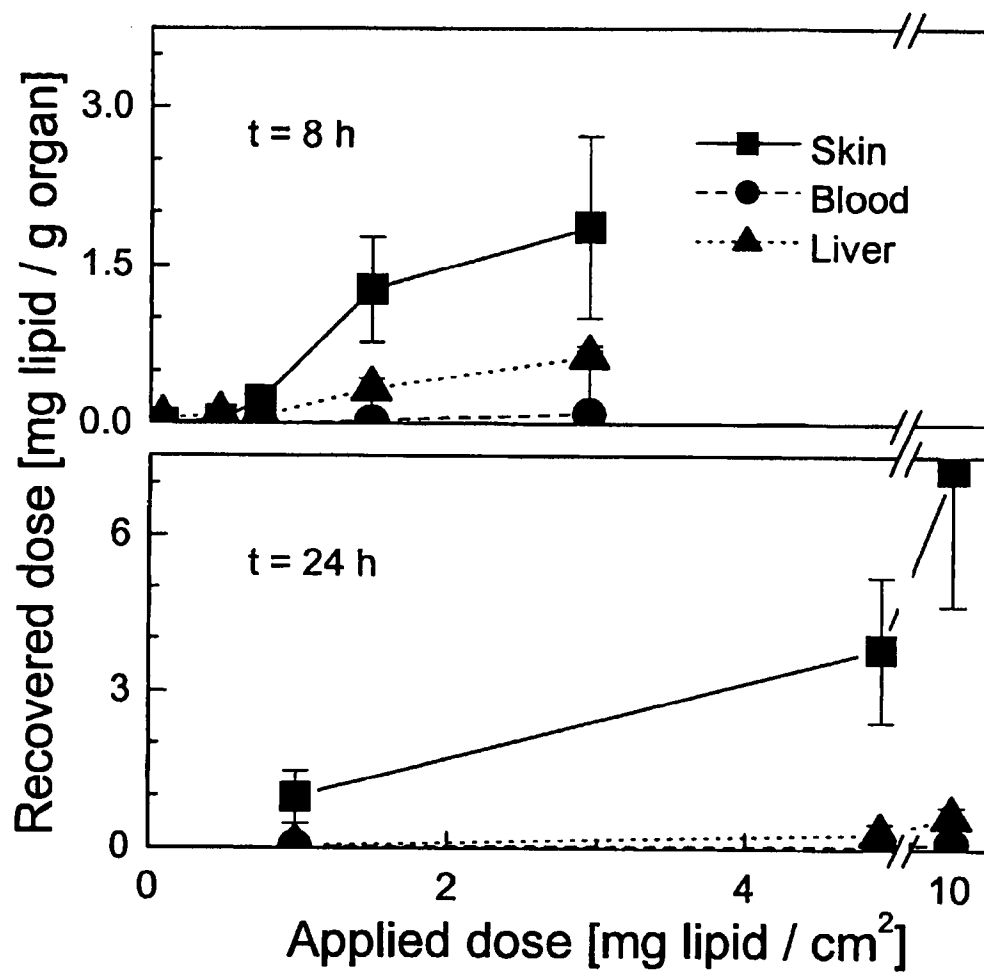


Figure 6

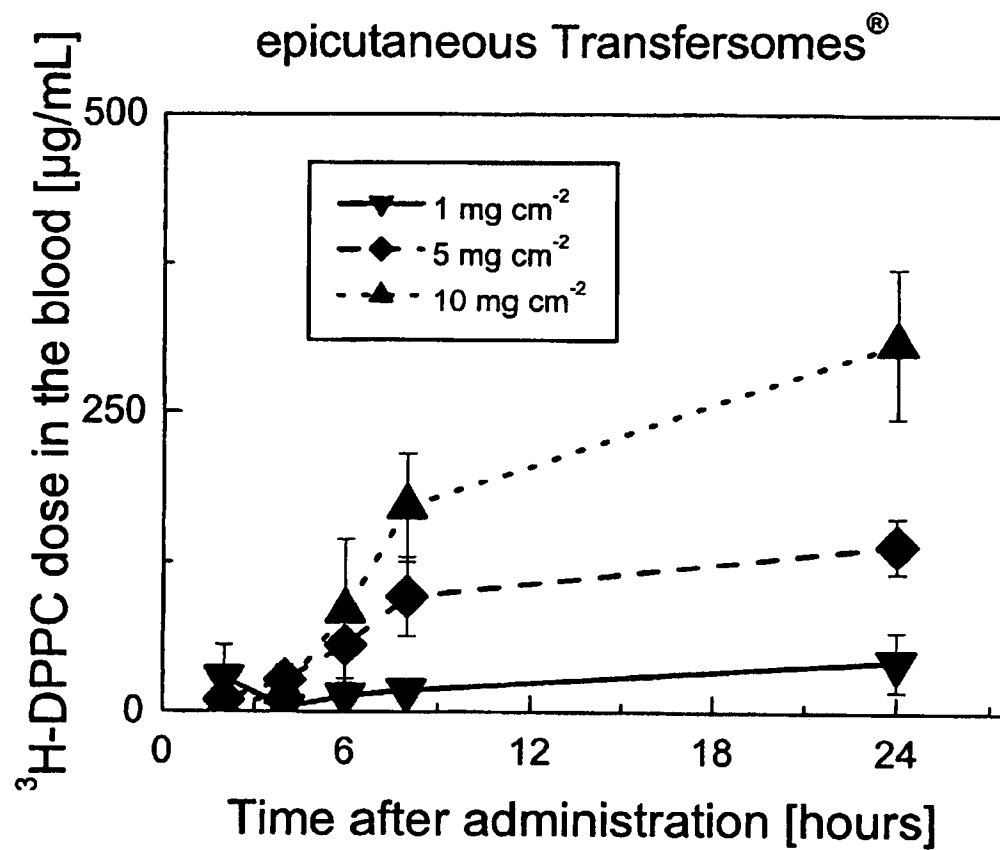
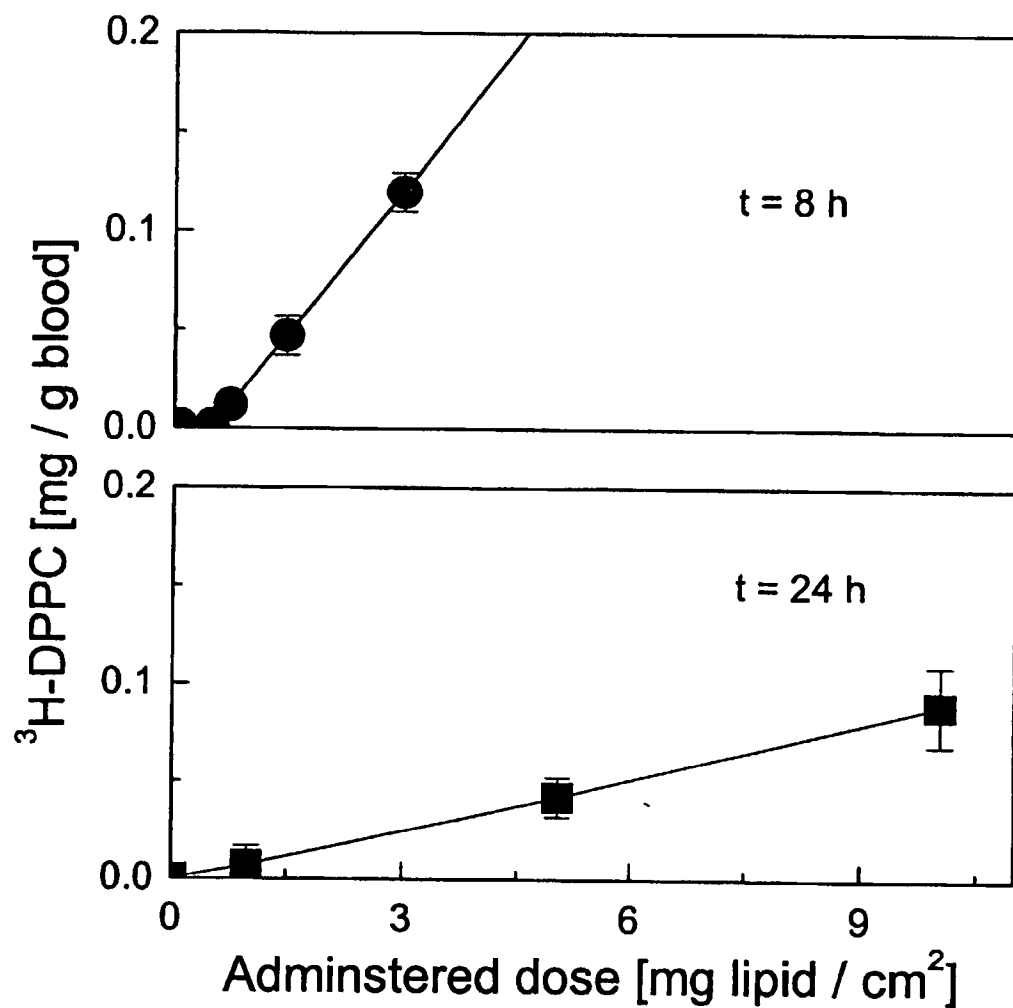


Figure 7



INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/EP 99/04659

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K9/127 A61K9/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>G. CEVC ET AL.: "transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo" JOURNAL OF CONTROLLED RELEASE, vol. 45, no. 3, 7 April 1997 (1997-04-07), pages 211-226, XP000640528 Amsterdam (nL) page 211, abstract page 225, conclusions page 213, paragraph 2.1.</p> <p style="text-align: center;">— —/—</p>	<p>1,2, 12-15, 18-29, 44,45, 55-59, 61-65, 68-71, 73,74</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search

9 March 2000

Date of mailing of the international search report

15/03/2000

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Benz, K

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 99/04659

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 17255 A (CEVC) 30 April 1998 (1998-04-30)</p> <p>the whole document page 19, line 21 - line 25 page 21, line 16 -page 23, line 17 page 24, line 9 - line 25 page 27, line 4 -page 28, line 4 claim 18</p>	<p>1-28, 44-47, 51,53, 55-59, 61-66, 68-74</p>
A	<p>V.M. KNEPP ET AL.: "controlled drug release from a novel liposomal delivery system. II. transdermal delivery characteristics" JOURNAL OF CONTROLLED RELEASE, vol. 12, no. 1, March 1990 (1990-03), pages 25-30, XP000113393 Amsterdam (NL) page 26, column 1, paragraph 6. page 26, column 2, paragraph 2</p>	29,31
A	<p>EP 0 674 913 A (LECTEC CORPORATION) 4 October 1995 (1995-10-04) the abstract</p>	1,29,32
A	<p>WO 98 30215 A (CILAG) 16 July 1998 (1998-07-16) claims 1-9</p>	4-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 99/ 04659

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 1-12, 15-28, 44-55, 58-71 (all partially) and 72-74 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/04659

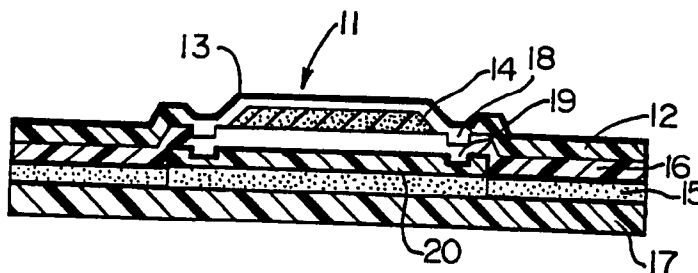
Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9817255 A	30-04-1998	AU 4510897 A EP 0935457 A	15-05-1998 18-08-1999
EP 674913 A	04-10-1995	US 5536263 A AU 676623 B AU 1002495 A CA 2133598 A FI 950465 A JP 7265353 A NO 951217 A US 5741510 A	16-07-1996 13-03-1997 12-10-1995 01-10-1995 01-10-1995 17-10-1995 02-10-1995 21-04-1998
WO 9830215 A	16-07-1998	AU 5777498 A	03-08-1998



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US90/01513 (22) International Filing Date: 21 March 1990 (21.03.90) (30) Priority data: 326,536 21 March 1989 (21.03.89) US (71) Applicant: THERATECH, INC. [US/US]; Research Park, 410 Chipeta Way, Suite 219, Salt Lake City, UT 83108 (US). (72) Inventors: CHANG, Yunik ; 601 Branch Drive, Toms River, NJ 08753 (US). PATEL, Dinesh, C. ; 5839 South Meadowcrest Drive, Murray, UT 84107 (US). EBERT, Charles, D. ; 558 North Perry's Hollow, Salt Lake City, UT 84103 (US).	(74) Agents: CIOTTI, Thomas, E. et al.; Irell & Manella, 545 Middlefield Road, Suite 200, Menlo Park, CA 94025 (US). (81) Designated States: AT (European patent), AU, BE (Euro- + pean patent), CA, CH (European patent), DE (Euro- pean patent), DK (European patent), ES (European pa- tent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>	

(54) Title: DEVICE FOR ADMINISTERING AN ACTIVE AGENT TO THE SKIN OR MUCOSA



(57) Abstract

A transdermal drug delivery device (11) comprising a drug formulation-containing reservoir (13) defined by a backing layer (12) and a drug-permeable membrane layer (16), a peelable inner liner (20) that underlies the reservoir and a portion of the backing/membrane outwardly of the reservoir periphery, an adhesive layer (15) that underlies the inner liner and outwardly extending portions of the membrane/backing layers, and a peelable release liner layer (17) that underlies the adhesive layer with a first permanent heat seal (18) between the backing and the membrane about the perimeter of the reservoir and another peelable (impermanent) heat seal (19) between the membrane and the inner liner underlying the first permanent heat seal, the heat seals and peelable barrier layer providing barriers that isolate the drug formulation from the adhesive.

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5

DEVICE FOR ADMINISTERING AN ACTIVE AGENT
TO THE SKIN OR MUCOSA

10

Description

Technical Field

This invention is in the field of transdermal/
15 transmucosal administration of active agents (drugs).
More particularly it relates to a device for achieving
such administration comprising an active agent-containing
reservoir and an adhesive layer for affixing the device to
the skin or mucosa in which the adhesive layer is peri-
20 pheral to the path of the active agent to the skin or
mucosa and is protected from degradation by the components
of the reservoir by a multiplicity of heat seals.

Background of the Invention

25 There are many patents describing devices for
administering drugs through the skin or mucosa. These
devices are commonly in the form of a laminated composite
that includes a reservoir layer containing the drug, a
pressure sensitive adhesive layer for attaching the
30 composite to the skin, and a backing layer that forms the
upper layer of the device. Depending upon the particular
drug and drug formulation involved, the reservoir layer
may be a matrix in which the drug formulation is dispersed
or a layer in the form of a walled container which holds
35 the drug formulation. Container-type reservoirs are often

formed as a pocket between the backing layer and a drug-permeable basal membrane through which the drug passes to the skin. The pressure sensitive adhesive layer normally underlies the membrane and the drug also passes through it
5 on its way to the skin.

Devices having container-type reservoirs with underlying pressure sensitive adhesive layers have significant disadvantages when one or more components of the drug formulation that are released from the reservoir
10 to the skin are solvents for the adhesive or otherwise adversely effect the properties of the adhesive as they pass through it to the skin. In such cases those reservoir component(s) cannot be permitted to pass through the adhesive and means must be found to isolate the adhesive
15 from them. Further, in such devices the drug partitions into the adhesive and alters drug release characteristics over prolonged storage. The present invention provides a device design in which the adhesive is peripheral to the path of the drug formulation and is isolated from the drug
20 formulation by a peelable barrier disc and a multiplicity of heat seals between selected layers of the device.

At least one other transdermal drug delivery device design has been proposed which involves an adhesive layer that is peripheral to the path of the drug to the
25 skin. U.S. Patent No. 4,573,996 describes a device that has both a drug-permeable adhesive layer in the path of the drug and a peripheral drug-impermeable adhesive layer that is not in the path of the drug. The purpose of the peripheral adhesive layer is to provide a site for
30 handling the device which avoids the risks of altering the drug path or contaminating the fingers with drug. Figure 6 of the patent shows a multi-layer laminated composite composed of (1) a backing layer, (2) a drug permeable membrane underlying the backing that forms with the backing a
35 pocket that serves as a drug-containing reservoir, (3) a drug-permeable adhesive layer directly underlying the

membrane, (4) a ring-shaped drug-impermeable adhesive layer adjacent and peripheral to the drug-permeable adhesive layer, and (5) a basal removable protective layer. The combination of a heat seal between the backing and the
5 membrane at the edge of the reservoir and the peripheral drug-impermeable adhesive layer prevents radial or horizontal migration of the drug from the reservoir. This patented device is distinct from the device of the present invention in several respects. The patented device does
10 not involve the problem of keeping drug formulation components isolated from the adhesive layer. In the patented device, the drug passes through the drug-permeable adhesive layer. There is only a single heat seal shown in the patented device. And, the single heat
15 seal is not used to isolate the drug formulation from either adhesive layer.

The present invention is also unique in that it employs two peelable layers, a permanent heat seal and a peelable heat seal in a manner that permits the creation
20 of a peripheral ring of adhesive when the two peelable layers are removed from the device.

The presently claimed devices are variations of the devices described in U.S. Patent No. 4,849,224. They differ from the devices in one or both of (1) the relative
25 locations of the permanent and peelable heat seals and (2) the manner in which the first and second peelable layers are bonded together.

Disclosure of the Invention

30 The invention is a device for administering an active agent to the skin or mucosa of an individual comprising a laminated composite of:

- (a) a backing layer;
- (b) an active agent-permeable membrane, the
35 backing layer and membrane defining

- (c) a reservoir therebetween that contains a formulation of the active agent, said reservoir having a smaller periphery than the backing layer and membrane such that a portion of the backing layer and membrane extends
5 outwardly of the periphery of the reservoir;
- (d) a first peelable active agent formulation-impermeable layer that underlies the reservoir and a portion of said outwardly extending portion of the backing layer and membrane;
- 10 (e) an adhesive layer that underlies and covers the first peelable active agent formulation-impermeable layer and said outwardly extending portion of the backing layer and membrane;
- (f) a second peelable active agent formulation-impermeable layer that underlies and covers the adhesive
15 layer;
- (g) a permanent heat seal about the periphery of the reservoir between the backing layer and the membrane; and
- 20 (h) a peelable heat seal between the membrane and the first peelable active agent formulation-impermeable layer located underneath and at a radius not less than that of the permanent heat seal, said permanent and peelable heat seals providing barriers to migration of
25 components of the active agent formulation from the reservoir into the adhesive layer and said first and second peelable active agent impermeable layers being bonded together such that when the second peelable layer is removed from the device the peelable heat seal is
30 broken and the first peelable layer and underlying portion of the adhesive layer is removed therewith.

Brief Description of the Drawing

Figure 1 is an enlarged sectional view of one
35 embodiment of the invention.

Figure 2 is an enlarged sectional view of the embodiment of Figure 1 after the second and first peelable layers have been peeled off the remainder of the embodiment.

5 Figures 3 and 4 are enlarged sectional views of a portion of other embodiments depicting alternative means for affixing the first and second peelable layers together.

10 The drawings are not to scale and like parts are referred to by like reference numerals in the various figures.

Modes for Carrying Out the Invention

15 The drawing shows a device, generally designated 11, that is an embodiment of the invention that is designed to administer a formulation of a drug and/or a permeation enhancer that is a solvent for pressure sensitive adhesives that are commonly used in transdermal delivery devices. Device 11 is designed to place the
20 adhesive out of the path of the enhancer-drug formulation and to prohibit radial or horizontal migration of the drug/enhancer into the adhesive. Device 11 is a laminated composite. The uppermost layer of the composite is a heat-sealable backing film 12 having an inverted, cup-
25 shaped recess 13 that serves as a container or reservoir for a drug-enhancer formulation 14. Underlying the reservoir and all or a portion of the part of the backing layer outwardly of the reservoir is a membrane layer 16 that is permeable to the drug-enhancer formulation. An
30 inner peel sealable liner 20 underlies the membrane layer and extends outwardly of the periphery of the reservoir. The next layer in the composite is a pressure-sensitive adhesive layer 15 that underlies the inner peel sealable liner and the portion of the backing layer that extends
35 outwardly of the edge of the liner. Finally a peelable

adhesive release liner layer 17 covers the entire underside of the assembly and forms the basal surface of the device. There are a minimum of two concentric heat seals in the composite. The first is at 18 between the membrane and the backing. It extends completely around the perimeter of the reservoir and forms a permanent seal between the backing film and membrane. The second is at 19 and is between the outer edge of the inner peel sealable liner and the membrane and forms a peelable (impermanent) seal between the membrane and inner liner. It is underneath the first heat seal and at a radius not less than that of the first heat seal. In the embodiment shown in Figure 1, it is located vertically in line with the first heat seal. These seals prevent the drug/enhancer formulation from migrating into the adhesive during storage. After the release liner is removed, the first heat seal prevents such migration during wearing. The width of the seals will usually be in the range of 0.05 cm to 1.0 cm. The peel strength between the adhesive layer and the release liner layer is greater than the force required to break the peelable seal at 19. Thus, when the release liner is peeled from the underside of the assembly the peelable seal is broken and the adhesive layer peripheral to the inner peel sealable liner is cut by the edge of that liner as the release liner and peel sealable liner 20 are removed, leaving the portion of the adhesive between liners 17 and 20 and creating a peripheral ring of adhesive underlying the membrane and backing peripheral to the reservoir (see Figure 2). Alternatively, the release liner and the inner peel sealable liner may be bonded together (e.g., by permanent adhesive or mechanical bonding) such that removal of the release liner results in simultaneous removal of the inner liner. Figures 3 and 4 depict such alternative bonding means. These means are also described in Examples 5 and 6, infra. In Figure 3 the means is a metal staple 21 that passes vertically

through the first peelable layer 20, the underlying adhesive layer 15 and the second peelable (release) layer 17 just inwardly of the edge of layer 20. Correspondingly, in Figure 4 the means is a plastic rivet 22 that is similarly passed through the three mentioned layers.

When device 11 is placed into use, the release liner layer 17 and inner liner 20 are peeled away from the underside of the device and discarded. This operation directly exposes the undersurfaces of the membrane and the peripheral ring of adhesive layer and the device can be placed on a desired site on the skin or mucosa of the individual to be treated with the active agent.

In the embodiment shown in Figures 1 and 2 the second impermeable heat seal is formed between the membrane and inner liner. It will be appreciated in this regard that additional heat-sealable layers could be included in the device between any of the component layers that are part of the membrane, backing or inner liner, as the case may be.

The invention device is useful when one or more of the components of the active agent formulation is incompatible with available adhesives that are useful for removably attaching elements to the skin or mucosa. The term "incompatible" is intended to mean that through physical and/or chemical interaction of the component(s) with the adhesive the adhesiveness or other desirable properties (e.g., nonirritancy) of the adhesive are significantly destroyed or impaired. The drug itself may be such a component or a carrier, solvent, skin permeation enhancing agent or other additive may be such a component. Also, this design prevents migration of drug into the adhesive which otherwise alters drug release characteristics over prolonged storage.

The backing layer 12 of the device may be composed of a single film or a plurality of films. In any

event, its inner surface must be capable of being heat sealed to the membrane. One or more of the films that constitute the layer will be impermeable to components of the drug formulation contained in the reservoir. Examples
5 of materials used as backing layers in transdermal delivery devices that may find use in the present invention are polyethylene, polypropylene, polyethylene vinylacetate, polyethylene terephthalate, and combinations thereof. The layer may include one or more metal layers
10 and/or one or more fibrous layers.

The reservoir pocket in the backing may be formed by vacuum forming or other like methods of forming desired shapes in films.

The term "drug" as used to describe the
15 principal active ingredient of the device intends a biologically active compound or mixture of compounds that has a therapeutic, prophylactic or other beneficial pharmacological and/or physiological effect on the wearer of the device. Examples of types of drugs that may be used in
20 the invention device are antiinflammatory drugs, analgesics, antiarthritic drugs, antispasmodics, antidepressants, antipsychotic drugs, tranquilizers, antianxiety drugs, narcotic antagonists, antiparkinsonism agents, cholinergic agonists, anticancer drugs,
25 immunosuppression agents, antiviral agents, antibiotic agents, appetite suppressants, antiemetics, anticholinergics, antihistamines, antimigraine agents, coronary, cerebral or peripheral vasodilators, hormonal agents, contraceptive agents, antithrombotic agents,
30 diuretics, antihypertensive agents, cardiovascular drugs, and the like. The appropriate drugs of such types are capable of permeating through the skin either inherently or by virtue of treatment of the skin with a percutaneous absorption enhancer. Because the size of the device is
35 limited for patient acceptance reasons, the preferred drugs are those that are effective at low concentration in

the blood stream. Examples of specific drugs are steroids such as estradiol, progesterone, norgestrel, levonorgestrel, norethindrone, medroxyprogesterone acetate, testosterone and their esters, nitro-compounds such as
5 nitroglycerine and isosorbide nitrates, nicotine, chlorpheniramine, terfenadine, triprolidine, hydrocortisone, oxycam derivatives such as piroxicam, ketoprofen, mucopolysaccharidases such as thiomucase, buprenorphine, fentanyl, naloxone, codeine, dihydroergotamine, pizo-
10 tiline, salbutamol, terbutaline, prostaglandins such as misoprostol and enprostil, omeprazole, imipramine, benzamides such as metoclopramine, scopolamine, peptides such as growth releasing factor and somatostatin, clonidine, dihydropyridines such as nifedipine, verapamil,
15 ephedrine, pindolol, metoprolol, spironolactone, nicardipine hydrochloride, calcitriol, thiazides such as hydrochlorothiazide, flunarizine, sydononimines such as molsidomine, sulfated polysaccharides such as heparin fractions and the salts of such compounds with pharma-
20 ceutically acceptable acids or bases, as the case may be.

Depending upon the inherent permeability of the skin to the particular drug or drugs being administered by the device, the reservoir may also contain a percutaneous absorption enhancer that increases the permeability of the
25 skin to the drug(s) and is coadministered to the skin. Examples of percutaneous absorption enhancers are those referred to in U.S. Patents Nos. 3,989,816, 4,316,893, 4,405,616, 4,060,084, and 4,379,454 and J Pharm Sci (1975) 64:901-024. The formulation contained in the reservoir
30 may also include solvent(s), gelling agents, stabilizers, and other additives. As indicated previously one or more of these components or a combination of these components is incompatible with the adhesive.

The membrane is permeable to the drug. It may
35 be a "dense" membrane made of a material that is inherently permeable to the components of the reservoir that

are to be administered to the skin or mucosa or it may be made of a microporous material whose pores are filled with a drug-permeable material including the drug-enhancer formulation itself. In the case of dense membranes, the component(s) dissolve in the material and diffuse through the material to the skin. In the case of microporous materials the component(s) diffuse through the pores to the skin. The membrane may or may not be a rate-controlling element depending upon the particular drug involved, the permeability of the skin to the drug, and the rate of delivery required to provide therapy. Examples of materials for making dense membranes are given in U.S. Patents Nos. 3,598,122 and 4,650,484. Examples of materials for making microporous membranes are provided in U.S. Patents Nos. 3,797,494 and 4,031,894.

The adhesive layer is composed of a pressure sensitive surgical adhesive such as those that are commonly used to affix transdermal drug delivery devices, bandages or other dressings to the skin. Examples of such adhesives are polyisobutene, natural rubber adhesives, acrylic and methacrylic adhesives, and silicone adhesives.

The release liner layer 17 and inner liner 20 may be composed of a single layer or a multiplicity of layers. They should be (1) impermeable to the components of the drug formulation that diffuse through the membrane, (2) heat-sealable in the case of the inner liner, and (3) inherently strippable or peelable or rendered so by techniques such as silicon or fluorocarbon treatment or surface treatment with a seal incompatible layer. An example of a film having such properties is Bertek 4418 Peelable Seal.

The respective components of the device may be formulated and assembled using procedures that are known in the drug formulation, transdermal device, and laminating arts. The shape of the device is not critical, and devices of preformed shapes may be assembled directly or

punched, cut, or otherwise formed from large sheets of laminated composite.

The following examples further illustrate the invention. These examples are not intended to limit the
5 invention in any manner.

Examples

Example 1

10 A silicone adhesive is prepared by mixing Dow Corning 355 Medical Adhesive with Dow Corning 360 Medical Fluid (10,000 cps) to provide 20% (wt/wt) Medical fluid in the final adhesive. The adhesive/medical fluid mixture is coated onto an Akrosil Biorelease release liner using a
15 10 mil gap casting knife and the adhesive solvent is evaporated at 80°C for 15 min to provide a final dry adhesive coating thickness of 0.0025 inches. A peelable heat seal disc (Bertek 4418) is then die cut into a 1.375 inch diameter circular disc which is positioned onto the
20 adhesive surface of above adhesive-coated release liner with the peelable heat seal surface facing outward. A 0.002 inch thick microporous membrane (3M, MSP-61588) is then laminated over the entire surface of the above adhesive/release liner/peelable disc structure to form a
25 membrane/peelable disc/adhesive/release liner laminate (L1).

The backing film (Scotchpak 1012) is pressure formed to provide a 5 cm² surface area and a 0.4 cc volume circular shaped cup.

30 A gelled calcitriol/enhancer reservoir formulation is prepared by mixing sufficient amounts of calcitriol and Klucel HF[®] with a 67.5%/21.75%/7.5%/3.25% (volume percent) mixture of ethanol/water/glycerine/methyl laurate to provide a 100 ug/ml calcitriol concentration
35 and a 1.5% Klucel HF[®] gel.

To fabricate a clacitriol system, 0.4 ml of the gelled calcitriol formulation is pipetted onto the microporous membrane surface of the L1 laminate coinciding with the exact center of the peelable disc underlying the membrane. The backing film is then placed over the L1 laminate such that the pre-formed cup on the backing film is situated over the drug/enhancer gel. The backing film is then heat sealed to the L1 laminate using a 0.9934 inch diameter circular heat seal die with a 0.0787 inch width heat sealing zone at 320°C with 30 PSI pressure for 0.5 seconds. The single heat sealing step creates the permanent heat seal between the backing film and microporous membrane layers, and simultaneously forms the peelable seal between the microporous membrane and the peelable disc directly underneath the permanent seal.

The backing film is then sealed to the microporous membrane in the outer area peripheral to the drug-enhancer reservoir with a heated plate. Finally, a 20 cm² (overall surface area) calcitriol system is die cut from the heat sealed structure using a steel rule die.

The peel force between the silicone adhesive and the release liner is greater than the force necessary to break the peelable seal between the membrane and the peelable disc. Therefore, when the release liner is peeled away from the system, the peelable disc is removed with the release liner exposing the 5 cm² microporous membrane drug-enhancer delivery surface area and creating the peripheral adhesive pattern. The in vitro steady state calcitriol skin flux is determined using the methods of Merritt and Cooper (J. Controlled Release 1:161, 1984) to be 1 ug/cm² day.

Example 2

A membrane/peelable disc/adhesive/release liner laminate (L1) is prepared as described in Example 1 using a Scotchpak 1022 release liner in place of the Akrosil Biorelease release liner.

A pindolol-enhancer gel formulation is prepared by mixing adequate quantities of pindolol HCl and Klucel HF[®] with a mixture consisting of 50%/39%/10%/1% (volume percent) ethanol/water/glycerine/glycerol monooleate to provide a gel with a final pindolol concentration of 65 mg/cc and Klucel level of 1.5% (wt/wt).

The pindolol-enhancer gel is pipetted (0.4 ml) onto the L1 laminate and a Scotchpak 1012 backing film (0.4 ml cup previously formed) is positioned over the laminate. The backing film is then heat sealed to the L1 laminate and a final system is die cut as described in Example 1. When the release liner is peeled from the system, the peel force between the adhesive and release liner is greater than the force necessary to break the peelable seal between the peelable disc and the micro-porous membrane. The peelable disc is thus removed from the system with the release liner, creating the peripheral adhesive and exposing the drug-enhancer delivery surface area. The in vitro pindolol skin flux from the system is determined using the methods of Merritt and Cooper, supra, to be 33 ug/cm²/hr.

Example 3

An L1 laminate is prepared as described in Example 1 using a polyisobutylene (PIB) adhesive in place of the silicone adhesive and a Daubert C-150 release liner in place of the Akrosil Biorelease release liner. A nicardipine-enhancer gel formulation is prepared by mixing adequate quantities of nicardipine HCl and Klucel HF[®] with a 65%/10%/20%/5% (volume percent) mixture of ethanol/water/glycerine/glycerol monooleate to provide a final gel

with a nicardipine concentration of 150 mg/cc and a Klucel level of 1.5% (wt/wt). A nicardipine transdermal system is then prepared as described in Example 1 using the nicardipine-enhancer gel formulation.

5 As with the previous examples, the peel force between the PIB adhesive and the release liner is greater than the force necessary to break the peelable seal between the microporous membrane and the peelable disc. As such, the peelable disc is removed with the release
10 liner when the release liner is peeled away from the system, simultaneously creating the peripheral adhesive pattern. The in vitro skin flux from the nicardipine system is determined using the methods described above to be 15 ug/cm²/hr.

15

Example 4

The L1 laminate is prepared as described in Example 1 using 3M #93088 medical grade acrylic adhesive in place of the silicone adhesive and a silanized release
20 liner in place of the Akrosil Biorelease release liner.

Prior to laminating the microporous membrane, the disc is fastened to the underlying release liner by using a sewing needle with a nylon thread. The needle with the nylon thread is pushed through the disc at a
25 distance of 0.0469 inches from its peripheral edge through the underlying adhesive and release liner. This procedure is repeated in the opposite direction by first piercing the release liner followed by the disc 0.1875 inches removed from the first stitch, while still maintaining 1
30 mm distance to the edge of the disc. The nylon thread is pulled tight and the two ends are tied to each other forming a knot as close to the surface of the disc as possible. This stitch forms the mechanical bond between the disc and the release liner.

35 The 0.002 inch thick microporous membrane (3M MSP-61588) is then laminated over the entire surface of

the above peelable disc/adhesive/release liner structure to form a membrane/peelable disc/adhesive/release liner laminate. This structure is used to fabricate calcitriol, pindolol and nicardipine transdermal systems as described in Examples 1, 2 and 3.

Example 5

An L1 laminate is prepared as described in Example 4 except that a mechanical bonding of the disc to the release liner is obtained by stapling the disc to the release liner. The disc is stapled .030 of an inch removed from the peripheral edge of the disc to the release liner by using a 0.375 inch long metal staple. Calcitriol, pindolol and nicardipine transdermal systems are then prepared as described in Examples 1, 2 and 3.

Example 6

An L1 laminate is prepared as described in Example 4 except that the mechanical bond is obtained by the use of a plastic rivet. This rivet is formed by first punching a 0.020 inch diameter hole into the disc/adhesive/release liner laminate. The center of this hole is 0.030 inches set back from the edge of the disc.

A thermoset polymer is then extruded into this hole and forms a mechanical bond upon cooling.

Transdermal systems are then prepared from this L1 laminate as described in the previous examples.

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Claims

1. A device for administering an active agent
5 to the skin or mucosa of an individual comprising a laminated composite of:
- (a) a backing layer;
 - (b) an active agent-permeable membrane, the backing layer and membrane defining
 - 10 (c) a reservoir therebetween that contains a formulation of the active agent, said reservoir having a smaller periphery than the backing layer and membrane such that a portion of the backing layer and membrane extends outwardly of the periphery of the reservoir;
 - 15 (d) a first peelable active agent formulation-impermeable layer that underlies the reservoir and a portion of said outwardly extending portion of the backing layer and membrane;
 - (e) an adhesive layer that underlies and covers
 - 20 the first peelable active agent formulation-impermeable layer and said outwardly extending portion of the backing layer and membrane;
 - (f) a second peelable active agent formulation-impermeable layer that underlies and covers the adhesive
 - 25 layer;
 - (g) a permanent heat seal about the periphery of the reservoir between the backing layer and the membrane; and
 - (h) a peelable heat seal between the membrane
 - 30 and the first peelable active agent formulation-impermeable layer located underneath and at a radius not less than that of the permanent heat seal, said permanent and peelable heat seals providing barriers to migration of components of the active agent formulation from the
 - 35 reservoir into the adhesive layer and said first and second peelable active agent impermeable layers being

bonded together such that when the second peelable layer is removed from the device the peelable heat seal is broken and the first peelable layer and underlying portion of the adhesive layer is removed therewith.

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2. The device of claim 1 wherein the adhesive is incompatible with one or more of the components of the formulation that permeate through the membrane to the skin or mucosa.

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3. The device of claim 1 wherein the backing layer is a laminated composite of at least one layer that is impermeable to the formulation and an inner heat-sealable layer.

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4. The device of claim 1 wherein the adhesive is an acrylic adhesive, the active agent is pindolol hydrochloride, and the formulation includes ethyl alcohol and glycerol monooleate.

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5. The device of claim 1 wherein the adhesive is an acrylic adhesive, the active agent is nicardipine hydrochloride, and the formulation includes ethyl alcohol and glycerol monooleate.

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6. The device of claim 1 wherein the adhesive is a silicone adhesive, the active agent is calcitriol and the formulation includes ethanol, methyl laurate, and water.

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7. The device of claims 1, 2, 3, 4, 5 or 6 wherein the peelable heat seal is located vertically in line with the permanent heat seal.

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8. The device of claims 1, 2, 3, 4, 5, 6 or 7 wherein the first and second peelable active agent impermeable layers are bonded together with a permanent adhesive or by mechanical bonding.

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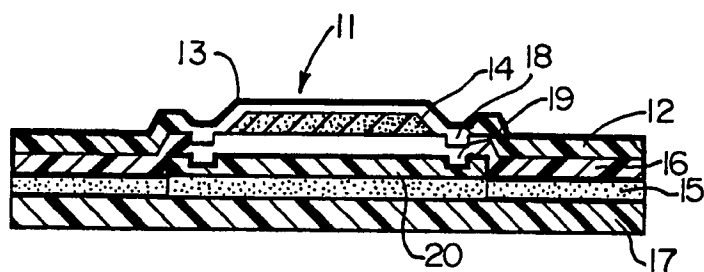


FIG. 1

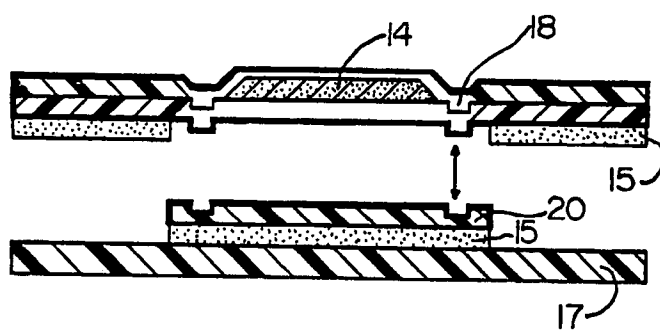


FIG. 2

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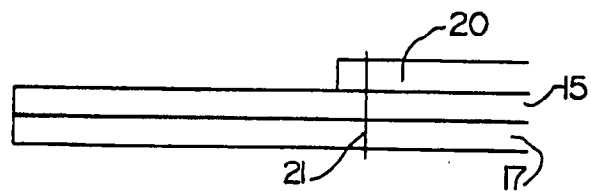


FIG. 3

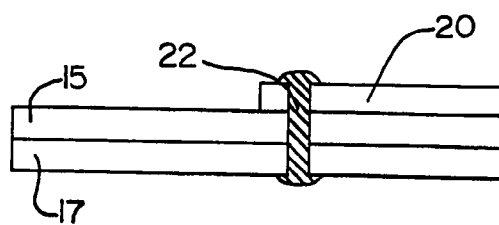


FIG. 4

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US90/01513

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

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IPC (5) : A61F 13/02

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II. FIELDS SEARCHED

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U.S.

424/448, 449

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X,P	US,A 4,849,224 (CHANG ET AL) 18 July 1989 See Abstract, Column 2, Lines 12-35.	1-8
A	US,A 4,696,821 (BELSOLE) 29 September 1987 See Abstract.	1-8
A,P	US,A 4,900,554 (XANAGIBASHI ET AL) 13 February 1990, See Column 2, Lines 7-21.	1-8

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

01 MAY 1990

Date of Mailing of this International Search Report

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Leon Horne



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/20261 (22) International Filing Date: 3 September 1999 (03.09.99) (30) Priority Data: 09/146,395 3 September 1998 (03.09.98) US (63) Related by Continuation (CON) or Continuation-In-Part (CIP) to Earlier Application US 09/146,395 (CON) Filed on 3 September 1998 (03.09.98) (71) Applicant (for all designated States except US): LOMA LINDA UNIVERSITY MEDICAL CENTER [US/US]; Room LLUMC - A505, 11234 Anderson Street, Loma Linda, CA 92354 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): WECHTER, William, J. [US/US]; 2285 East Ojai Avenue, Ojai, CA 93023 (US). (74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, LLP, 16th Floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).		(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PHARMACEUTICAL COMPOSITION AND METHOD FOR TREATMENT OF INFLAMMATION (57) Abstract <p>The present invention relates to pharmaceutical compositions comprising enantiomerically pure R-NSAIDs and the methods of their use for the treatment of inflammation. Preferably, the R-NSAID used is R-flurbiprofen which is administered in a dose of at least 2.5 milligrams per kilogram of body weight per day. The anti-inflammatory action of R-NSAIDs is due to their ability to interfere with the biosynthesis of COX-2 by inhibiting COX-2 mRNA synthesis, rather than by just blocking the action of the enzyme itself. In order to effect the inhibition of COX-2 mRNA synthesis, the R-NSAID must be present at relatively high doses. Because the R-NSAID is selective in its action, that is it does not inhibit either COX-1 mRNA synthesis or the COX-1 enzyme itself, it can be administered in the required high doses because the tissue protective effects of prostaglandins made through the COX-1 pathway are not interfered with.</p>		

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PHARMACEUTICAL COMPOSITION AND METHOD FOR TREATMENT OF INFLAMMATION

Field of the Invention

The present invention relates to pharmaceutical compositions comprising enantiomerically pure R-NSAIDs and the methods of their use for the treatment of inflammation. Preferably, the R-NSAID used is R-flurbiprofen which is administered in a dose of at least 2.5 milligrams per kilogram of body weight per day.

Background of the Invention

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been in use for over a century beginning with aspirin. In recent decades the arylpropionic acid (APA) class of these drugs has gained wide acceptance.

Although the NSAIDs are known to be effective against pain and inflammation, there are often severe side effects and toxicity associated with chronic use of these drugs. Chronic NSAID use is known to cause gastric and duodenal ulceration, which may be severe enough to result in significant morbidity and mortality. Furthermore, NSAID use has been reported to be associated with renal and hepatic toxicities, increase in bleeding times due to disruption of platelet function, prolongation of gestation due to uterine effects, and a decreased white cell count in the blood. Because of the side effects and toxicity, many NSAIDs are no longer in use in human medicine as analgesics. Some of these include tiaprofenic acid, suprofen, carprofen, pirofen, benoxaprofen, and indoprofen.

Some NSAIDs, including the APAs, exhibit molecular chirality and thus have R- and S-enantiomers. The APAs, with the exception of naproxen, are currently prescribed as racemates.

For a given NSAID, there can be a difference in the properties exhibited by the R- and S-enantiomers. One important difference relates to the activities of the two enantiomers in connection with prostaglandin synthesis.

Prostaglandins are autoids, produced by the body, which serve a variety of functions. An important step in the biosynthesis of prostaglandins requires the use of two cyclooxygenase (COX) enzymes, COX-1 or COX-2. COX-1 is present throughout the body and makes the prostaglandins that, among other things, help keep the stomach lining intact and the aid proper function of the kidneys. COX-2 is made by the body only under certain conditions, such as in response to tissue injury, and the prostaglandins produced by it are associated with pain and inflammation.

Researchers found that the S-enantiomers of NSAIDs were much better at inhibiting prostaglandin synthesis than the R-enantiomer, having 15-100 or even 500 times higher prostaglandin synthetase inhibitory activities than the R-enantiomers in the rat. Yamaguchi et al., Nippo Yakurigaku Zasshi, 90:295-302 (1987). Thus, it was thought that the biological activity of NSAIDs resided principally if not only in the S-enantiomers. Some researchers went as far as to say that "at best, the R-isomers [of APAs] function as prodrugs for the therapeutically active S-forms" when the racemic drug is administered to the host, and that the R-enantiomers are "undesirable impurities in the active drug." Caldwell et al., Biochem. Pharmacol. 37:105-114 (1988).

Although the S-NSAIDs have the desired effect of inhibiting production of prostaglandins through the COX-2 pathway, they also inhibit the production through the COX-1 pathway and thus the bad side effects of NSAID use generally are also associated with the use of S-enantiomers.

Earlier studies by researchers in this field, as well as by the inventor himself, found that R-NSAIDs had little or no inhibiting effect on COX enzymes and prostaglandin production. What little anti-inflammatory effect existed was either found to be statistically insignificant or attributed to the S-enantiomer, the presence of which was due to either an enantiomerically impure dose of R-NSAID or inversion of the R-enantiomer *in vivo*. See K. Brune et al, Pure Enantiomers of 2-Arylpropionic Acids: Tools in Pain Research and Improved Drugs in Rheumatology, *J. Clin. Pharmacol.* 32:944-52, 946
5 (1992); K. Brune et al., Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition, *Experientia* 47:257-61, 260 (1991); U.S. Patent No. 5,200,198 to Geisslinger et al.; and U.S. Patent No. 5,206,029 to Brune et al.

Although the researchers did not find significant anti-inflammatory activity, there was evidence of other activity attributable to the R-enantiomer, such as amelioration of pain (see U.S. Patent Nos. 5,200,198 and 5,206,029), treatment
10 and prevention of cancer (see Wechter et al., R-Flurbiprofen Chemoprevention and Treatment of Intestinal Adenomas in the APC^{min}/+ Mouse Model, *Cancer Research* 57:4316-24 (1997)), treatment of cystic fibrosis (see U.S. Patent Application Serial No. 09/058,093) and treating or delaying the onset of Alzheimer's Disease (see U.S. Patent Application Serial No. 08/814,490).

U.S. Patent Nos. 5,200,198 and 5,206,029 disclose the use of mixtures of R- and S-flurbiprofen to treat
15 diseases characterized by pain and/or inflammation. The inventors state that R-flurbiprofen has better pain amelioration activity than S-flurbiprofen, and that the known side effects are coupled with the anti-inflammatory effects in S-flurbiprofen. Thus, according to the inventions, if one were to create a medicament for the treatment of a disease that was characterized primarily by pain, one would use a mixture having an excess of the R-flurbiprofen, so as to maximize the effects against pain. The amount of S-flurbiprofen in the mixture would be minimized, so as to attain a balance between
20 the needed anti-inflammatory activity and the undesired side effects which result from use of the S-enantiomer. If, according to the disclosures of the '198 and '029 patents, one wanted to create medicament for the treatment of a disease characterized by both pain and inflammation, one would increase the amount of S-flurbiprofen in the composition in order to have the needed anti-inflammatory effect and would have to accept the unwanted side effects.

A common test which is used to determine whether a compound has activity as an anti-inflammatory drug is the
25 carrageenan paw test. In this test, the test compound is administered to several rats. Thereafter, a paw on each rat is injected with a solution of carrageenan in order to induce edema in the paw as a measure of inflammation. After three hours, the volume of the paw is measured using a plethysmometer. Reduction of edema by 30% or more, as compared to a control group which was not given the test compound, is considered indicative of anti-inflammatory activity.

In the '198 and '029 patents, there is described the results of the carrageenan paw test done using a dosage of
30 0.3 mg/kg of R- or S- flurbiprofen. The enantiomeric purity of the compounds used is not disclosed. For these tests, it was reported that the reduction of edema for the S-flurbiprofen was 64%, indicating anti-inflammatory activity, but for the R-flurbiprofen it was only 18% which does not indicate anti-inflammatory activity.

The inventors in the '198 and '029 patents, Brune and Geisslinger, later reported carrageenan paw tests using dosages of approximately 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, and 2.5 mg/kg of R- and S-flurbiprofen (*Experientia*, 47:257-261).
35 Only at the highest dose, 2.5 mg/kg, did the R-flurbiprofen demonstrate a statistically significant reduction in inflammation.

In a 1996 article entitled New Insights into the Site and Mode of Antinociceptive Action of Flurbiprofen Enantiomers (J. Clin. Pharmacol. 36:513-20), Brune and Geisslinger discussed the tests first reported in the Experientia article, stating that: "as expected, only the S-enantiomer had antiinflammatory activity. The antiinflammatory effects after administration of higher doses of R-flurbiprofen may be explained by an S- impurity of the administered R-enantiomer (purity: S-flurbiprofen, 98.5%; R-flurbiprofen, 99.1%) and/or by small amounts of S-flurbiprofen formed by inversion."

More recently, following the discovery of the differences between COX-1 and COX-2, some drug companies have set forth to make compounds which selectively inhibit COX-2, so as to achieve the desired anti-inflammatory and analgesic effects while avoiding the toxic effects associated with COX-1 inhibition. Some of these recent advances were discussed in a recent article in Science (Elizabeth Pennisi, "Building A Better Aspirin", Science 280:1191-92 (1988), and have even found their way to the mainstream media such as Jerome Groopman's article entitled "Superaspirin" (The New Yorker p.32-35 (1998)). These articles present the new COX-2 inhibitor drugs as far superior to the older NSAIDs and state that this new class of COX-2 inhibitors will eventually replace the older NSAIDs, which will "become dinosaurs." This current flurry of research and media activity makes it clear that a need remains for an anti-inflammatory compound characterized by an ability to selectively block prostaglandin production via the COX-2 pathway.

Summary of the Invention

There is provided in accordance with one aspect of the present invention, a method of treating inflammation in an animal. The method comprises administering to the animal a dose of at least 2.5 milligrams of an enantiomerically pure R-NSAID per kilogram of said animal's body weight. In other embodiments, the administered dose is at least 5 mg/kg, at least 25 mg/kg, and at least 50mg/kg of the animal's body weight. In preferred embodiments of the methods, the R-NSAID is selected from the group consisting of R-ketoprofen, R-flurbiprofen, R-ketorolac, R-etodolac, R-tiaprofenic acid, R-suprofen, R-carprofen, R-pirprofen, and R-benoxaprofen. In an especially preferred embodiment, the R-NSAID is R-flurbiprofen.

In accordance with a further aspect of the present invention, there is provided a pharmaceutical composition for the treatment of inflammation. The pharmaceutical composition comes in a unit dosage form and comprises at least 200 milligrams of an enantiomerically pure R-NSAID. In other embodiments, the unit dosage form is at least 400 milligrams, at least 1,000 milligrams, and at least 3,500 milligrams. In preferred embodiments, the R-NSAID of the pharmaceutical composition is selected from the group consisting of R-ketoprofen, R-flurbiprofen, R-ketorolac, R-etodolac, R-tiaprofenic acid, R-suprofen, R-carprofen, R-pirprofen, and R-benoxaprofen. In an especially preferred embodiment, the R-NSAID is R-flurbiprofen.

Detailed Description of the Preferred Embodiment

Although NSAIDs have been known for many years as anti-inflammatory drugs, the activity was believed to lie in the S-enantiomer only, due to its ability to inhibit the COX-2 enzyme. The R-enantiomer exhibits only minor inhibition of the COX-2 enzyme, and was thus believed to be inactive as an anti-inflammatory agent, though it was found to have utility for other indications.

It has surprisingly been found by the inventor herein that R-NSAIDs have anti-inflammatory activity when given at relatively high dosages, that is more than 2.5 mg/kg. The anti-inflammatory properties of R-NSAIDs is due to their ability to interfere with the biosynthesis of COX-2 by inhibiting COX-2 mRNA synthesis, rather than by just blocking the action of the enzyme itself. In order to effect the inhibition of COX-2 mRNA synthesis, the R-NSAID must be present at
5 relatively high concentrations. Because the R-NSAID is selective in its action, that is it does not significantly inhibit either COX-1 mRNA synthesis or either of the COX enzymes themselves, it can be administered in the required high doses because the tissue protective effects of prostaglandins made through the COX-1 pathway are not interfered with.

Because of the unpleasant and undesirable side effects associated with the S-enantiomers, it is desired that the R-NSAIDs used in the pharmaceutical compositions and methods of the present invention be substantially free of the S-
10 enantiomer, that is "enantiomerically pure." An enantiomerically pure R-NSAID, as that term is used herein, comprises preferably at least 98% R-NSAID, more preferably at least 99.5% R-NSAID, most preferably at least 99.9% R-NSAID.

For the methods and pharmaceutical compositions of the present invention, the enantiomeric purity is in reference to the R-NSAID as it exists prior to being administered to the animal host or patient. This is because both S- and R-arylpropionic acid NSAIDs are subject to interconversion by means of inversion when placed in vivo. The degree of
15 inversion varies widely among the NSAIDs and is generally different for the R- and S- enantiomeric forms of a given compound. The degree of inversion for a given enantiomer may also vary by the dose given, the species of animal tested, the gender of animal tested, and the amount of time the enantiomer has been in the animal's system.

Preferred R-NSAIDs for use in the methods and pharmaceutical compositions of the present invention are those which are enantiomerically stable. As used herein, "enantiomerically stable" means that at a steady state, there is
20 preferably no more than about 10% of the S-enantiomer of the NSAID in circulation, more preferably no more than 2%, most preferably no more than about 1% of the S-enantiomer in circulation, the S-enantiomer having been formed in vivo from inversion of the R-enantiomer. Examples of enantiomerically stable R-NSAIDs are R-flurbiprofen (1.5% S-enantiomer in circulation at steady state in humans), R-ketoprofen (10% S), R-ketorolac (6% S), and R-etodolac (0% S).

A large number of R-NSAIDs which are preferred for use in the medicaments and methods of the present
25 invention are commercially available. The enantiomeric purity of any given R-NSAID may vary among manufacturers. R-ketoprofen, R-flurbiprofen, and R-ketorolac are available through Sepracor, Inc. (Marlborough, MA); R-naproxen can be obtained as a sodium salt through Sigma Chemical Co.; R-etodolac is available from Wyeth-Ayerst; R-tiaprofenic acid is available through Roussel (France); R-suprofen is manufactured by McNeil Pharmaceuticals; R-carprofen is available from Roche (Switzerland); R-pirprofen is available through Carlo Erba (Italy); and R-benoxaprofen is manufactured by Eli Lilly and
30 Co. (Indianapolis, IN). Additionally, racemates which can be resolved by methods known in the art, may be obtained from several of the above sources.

The most preferred R-NSAID for use in the methods and pharmaceutical compositions of the present invention is R-flurbiprofen, based on its superior enantiomeric stability in humans and availability in a very enantiomerically pure form, up to 99.97%.

This very pure form of R-flurbiprofen, was tested for anti-inflammatory activity by the carrageenan paw test. Seven groups of eight male Long Evans derived rats weighing 150-200 grams were fasted overnight. One hour after oral administration a suspension of the test substance in 2% Tween 80, as documented in Table 1 below, the right hind paw of each rat was injected intraplantarly with 0.1 ml of a 1% suspension of carrageenan. Three hours after injection, the hind paw volume was measured using a plethysmometer and recorded.

Table 1.

RESULTS OF CARRAGEENAN PAW TEST			
Substance Tested	Dose	Result (Reduction of Edema)	Anti-Inflammatory Activity
Vehicle (2% Tween 80)	10 ml/kg	0%	No
Positive Control (Aspirin)	150 mg/kg	42%	Yes
R-Flurbiprofen	40 mg/kg	51%	Yes
R-Flurbiprofen	20 mg/kg	47%	Yes
R-Flurbiprofen	10 mg/kg	41%	Yes
R-Flurbiprofen	5 mg/kg	30%	Yes

As seen in Table 1 above, R-flurbiprofen present at high doses exhibits anti-inflammatory action, that is it causes a statistically significant reduction of edema in the rat.

The pharmaceutical compositions of the present invention can be prepared in any desired form, for example, tablets, powders, capsules, sterile suspensions or solutions for parenteral administration, non-sterile suspensions or solutions for oral administration, suppositories, aerosols, and the like. Furthermore, the pharmaceutical compositions of the present invention may be administered by any route including oral, intravenous, intramuscular, vaginal, rectal, topical, transdermal, buccal, nasal, inhalation, and the like. The use of controlled release means and other drug delivery devices are contemplated by the inventor.

In addition to one or more R-NSAIDs, the pharmaceutical compositions of the present invention may optionally comprise carriers, fillers, diluents, granulating agents, lubricants, binders, disintegrating agents, release agents and the like. Preferred fillers include starch, glucose, lactose, mannitol, calcium phosphate, calcium carbonate, and cellulose. Preferred lubricants include talc, calcium stearate, and magnesium stearate. Preferred release agents include carboxymethyl cellulose, carboxymethyl starch, polyvinylpyrrolidone (PVP), and silica gel. Which optional ingredients are present and the quantity used is dependent upon many factors, including the form the medicament will take, the desired strength of the

final composition, and the desired speed at which the active ingredients are to be released into the animal's system. It is within the abilities of one skilled in the art to create a suitable formulation for use in methods of the present invention, including the choice of optional ingredients and the amounts in which they are present.

5 The R-NSAIDs used in the methods and pharmaceutical compositions of the present invention may be present in the form of a pharmaceutically acceptable salt. When describing formulations used in the methods and compositions of the present invention, R-NSAID should be read as also including any of the pharmaceutically acceptable salts thereof. The term "pharmaceutically acceptable salt" as used herein refers to salts prepared from pharmaceutically acceptable, non-toxic acids or bases. Suitable pharmaceutically acceptable salts include inorganic salts, e.g. salts of aluminum, calcium, lithium, magnesium, potassium, sodium and zinc, or organic salts, e.g. salts of lysine, N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, procaine, and tris. A salt may be chosen to effect a particular rate of dissolution or uptake in the body, as is known in the art.

Of the available routes, the oral route is preferred, and oral solid preparations (e.g. tablets, capsules, caplets, powders, chewable tablets) are preferred over oral liquid preparations. The most preferred oral solid preparations are tablets. A common method of forming a suitable pressed tablet is to combine the powdered active material having a suitable particle size distribution with pharmaceutically compatible adjuvants, such as lubricants, inert diluents, dispersing agents, carriers binders, and the like, and then press the mixture in a suitable machine. Molded tablets may be made by molding the composition, which preferably comprises a mixture of powdered material comprising at least one R-NSAID and any optional adjuvants, moistened with an inert liquid diluent in a suitable molding machine as is known in the art. Molded tablets may also contain any optional material such as those described above. If the tablets are in chewable form, addition of one or more flavorings and sweeteners, such as saccharin, is preferred.

Another form for the oral route is the capsule. Capsules, preferably made of gelatin, may be filled with dry materials such as powder, granules and pellets or with a suspension such as that formed by mixing the R-NSAID with a material such as vegetable oil or other pharmaceutically compatible carrier.

Oral suspensions and solutions for use in the present invention preferably comprise at least one R-NSAID, water, sweetener (such as sugar, saccharin, or aspartame), a flavoring (such as mint, or any of the known FDA-approved artificial flavorings), and a suspension or emulsifying agent (such as Tween or tragacanth). Preferably such suspensions or solutions are made by first combining all ingredients other than the R-NSAID, and then mixing in a sufficient quantity of finely powdered R-NSAID to achieve a solution or suspension of desired strength.

Injectable solutions are preferably prepared by combining a salt of the R-NSAID with water or isotonic saline. Other materials such as preservatives, sugars, and other drugs may be added on an optional basis. After mixing, the solution is filtered and placed in a sterile container, such as a vial or plastic infusion bag. The concentration of the solution can vary widely, depending upon whether such solution is to be infused to a patient over time or administered via a single hypodermic injection.

The dose of R-NSAID may also take the form of a suppository for either rectal or vaginal administration. A suitable suppository composition comprises the active ingredient (R-NSAID) mixed with a carrier, such as a fat or

polyglycol, having a melting point at or near body temperature. Alternatively, the carrier may be a material which dissolves when placed in the rectum or vagina. The suppository is preferably made by mixing powdered R-NSAID with the carrier, and then forming the mixture into a generally cylindrical or bullet shape of a size which allows for insertion.

5 Other dosage forms can be prepared by one skilled in the art by the use of known or later developed techniques which allow for the administration of a solid substance to an animal.

The quantity of R-NSAID used in the pharmaceutical compositions and methods of the present invention is dependent upon the body weight of the animal to whom the drug is administered and the frequency of dosing. It is preferred that the total daily dose for anti-inflammatory effect be at least 2.5 milligrams of R-NSAID per kilogram of body weight of the animal (2.5 mg/kg), preferably at least 5 mg/kg, more preferably 25 - 50 mg/kg or more. As the compounds and compositions of the present invention are not toxic, it is not foreseen that there is an upper limit to the dose at which the compounds of the present invention can be given. A dose comprising several grams of an R-NSAID is contemplated by the inventor.

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The total quantity of dose may also be dependent upon the form which the dose takes and the relative efficiency or inefficiency of delivery of the drug by that particular dose form or route. For example, to achieve the same concentration of the drug in the body of the animal, it may be necessary to deliver a larger dose when a drug is administered via a relatively inefficient means such as a suppository or inhalation, as opposed to when the drug is administered by a more efficient method such as intravenous injection or orally.

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The preferred total daily dose may be administered in a single dose or in smaller doses administered two, three, four, or more times during the day which, when summed together, equal the total daily dose. If a drug delivery device such as a transdermal patch is used, the dose may be administered continually over a period of hours. The determination as to whether and to what extent the total daily dose should be broken down into smaller doses administered throughout the day is dependent on several factors, including the half-life of the particular R-NSAID in the body, the speed at which the form of the R-NSAID becomes biologically available, and the total quantity of R-NSAID which needs to be administered.

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Although the present invention has been described in terms of certain preferred embodiments, it is to be understood that the scope of the invention is not to be limited thereby. Instead, Applicant intends that the scope of the invention be limited solely by reference to the attached claims, and that variations on the formulation and dosages disclosed herein which are apparent to those of skill in the art will fall within the scope of Applicant's invention.

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WHAT IS CLAIMED IS:

1. A method of treating inflammation in an animal, comprising administering to said animal a dose of at least 2.5 milligrams of an enantiomerically pure R-NSAID per kilogram of said animal's body weight.
2. The method of Claim 1 wherein said dose is at least 5 milligrams of an enantiomerically pure R-NSAID per kilogram of said animal's body weight.
3. The method of Claim 1 wherein said dose is at least 25 milligrams of an enantiomerically pure R-NSAID per kilogram of said animal's body weight.
4. The method of Claim 1 wherein said dose is at least 50 milligrams of an enantiomerically pure R-NSAID per kilogram of said animal's body weight.
5. The method of Claim 1 wherein said R-NSAID is selected from the group consisting of R-ketoprofen, R-flurbiprofen, R-ketorolac, R-etodolac, R-tiaprofenic acid, R-suprofen, R-carprofen, R-pirprofen, and R-benoxaprofen.
6. The method of Claim 1 wherein said R-NSAID is R-flurbiprofen.
7. A pharmaceutical composition for the treatment of inflammation in a unit dosage form comprising at least 200 milligrams of an enantiomerically pure R-NSAID.
8. The pharmaceutical composition of Claim 7, wherein said unit dosage form comprises at least 400 milligrams.
9. The pharmaceutical composition of Claim 7, wherein said unit dosage form comprises at least 1,000 milligrams.
10. The pharmaceutical composition of Claim 7, wherein said unit dosage form comprises at least 3,000 milligrams.
11. The pharmaceutical composition of Claim 7, wherein said unit dosage form comprises at least 5,000 milligrams.
12. The pharmaceutical composition of Claim 7 wherein said R-NSAID is selected from the group consisting of R-ketoprofen, R-flurbiprofen, R-ketorolac, R-etodolac, R-tiaprofenic acid, R-suprofen, R-carprofen, R-pirprofen, and R-benoxaprofen.
13. The pharmaceutical composition of Claim 7 wherein said R-NSAID is R-flurbiprofen.



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(54) Title: TRANSDERMAL THERAPEUTIC SYSTEM CONTAINING ESTRADIOL (54) Bezeichnung: ESTRADIOLHALTIGES TRANSDERMALES THERAPEUTISCHES SYSTEM (57) Abstract <p>The invention concerns a transdermal therapeutic system designed for the controlled release of estradiol or any of its pharmaceutically acceptable derivatives, alone or in combination with gestagenes, the system comprising a back film, an active-substance reservoir which is joined to the back film and is produced using pressure sensitive adhesives, and a detachable protective film. The system is characterized in that the pressure sensitive adhesive contains esters of colophonium.</p> (57) Zusammenfassung <p>Ein wirkstoffhaltiges transdermales therapeutisches System zur kontrollierten Abgabe von Estradiol oder seinen pharmazeutisch unbedenklichen Derivaten allein oder in Kombination mit Gestagenen aus einer Rückschicht, einem damit verbundenen wirkstoffhaltigen Reservoir, das unter Verwendung von Haftklebern hergestellt ist und einer wiederablösbaren Schutzschicht, ist dadurch gekennzeichnet, daß der Haftkleber Ester des Kolophoniums enthält.</p>			

LEDIGLICH ZUR INFORMATION

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Estradiolhaltiges transdermales therapeutisches System

B E S C H R E I B U N G

Die Erfindung betrifft ein transdermales therapeutisches System zur kontrollierten Abgabe von Estradiol oder seinen pharmazeutisch unbedenklichen Derivaten alleine oder in Kombination mit Gestagenen wie Levonorgestrel an die menschliche oder tierische Haut, seine Verwendung und ein Verfahren zu seiner Herstellung.

Transdermale therapeutische Systeme (TTS) sind in der Therapie einer Reihe von Erkrankungen bereits im Markt eingeführt. Auch transdermale therapeutische Systeme mit dem estrogenen Wirkstoff 17- β -Estradiol sind als Therapeutikum für Wechseljahrsbeschwerden und neuerdings auch gegen Osteoporose im Handel und erfolgreich in der Therapie.

Levonorgestrel ist ein synthetisches Gestagenderivat, das in Kombination mit oral wirksamen Estrogenen bisher vor allem in Kontrazeptiva eingesetzt wurde. Gestagene, also auch Levonorgestrel, haben in solchen Präparaten die Funktion, durch entsprechende trophische Vorbereitung des Uterus eine möglichst kurze, schnelle und "physiologische" Entzugsblutung folgen zu lassen. Es gibt auch Hinweise auf eine Schutzwirkung der Gestagenzugabe vor dem Risiko endometrialer Tumoren.

Es ist daher sinnvoll, auch für die Indikation postmenopausaler Beschwerden zu einer zyklischen Behandlungsweise zu kommen d.h. zu einer zeitweisen festen Arzneimittelkombination aus Estrogenen (z.B. Estradiol) und Gestagenen (z.B. Levonorgestrel). Besonders attraktiv ist eine solche Kombination beider Wirkstoffe in einem gemeinsamen, monolithischen transdermalen therapeutischen

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System, das nur einmal am Tag oder sogar nur ein- bis zweimal pro Woche zu applizieren wäre. Aufgrund seiner hohen Wirksamkeit und Permeationsfähigkeit durch die Haut ist Levonorgestrel für ein solches System hervorragend geeignet.

In der Literatur sind experimentelle Systeme zur transdermalen Zufuhr von Levonorgestrel beschrieben (Friend et. al., J. Controlled Release 7, 243-250 (1988)). Allerdings sind nach dieser Einschätzung Permeationsverbesserer (Enhancer), z.B. Alkylester kurzfettiger Fettsäuren, zur erfolgreichen transdermalen Therapie mit ausreichend kleiner Systemfläche erforderlich (Friend et. al., J. Controlled Release 9, p33-40 (1989)).

Für die transdermale Anwendung von Estrogenen und Gestagenen sind bereits eine Reihe von Vorrichtungen bekannt geworden. Unter Verwendung von Styrol-Isopren-Blockcopolymer, feuchtigkeitsabsorbierenden Polymerdomänen und dem Enhancer (und Juckreizstiller) Crothamiton gelangten Nakagawa et al. (EP-A 0 483 370) zu einem Matrix-Transdermalen therapeutischen System für Estradiol allein. Eine andere Konzeption liegt in der gleichzeitigen Applikation von Estradiol und einem Enhancer (Ethanol) in einem membrangesteuerten Reservoirsystem (Campbell et al. US PS 4 379 454) vor, das auch in einer Kombinationsarzneiform mit dem Gestagen Norethisteronacetat verwendbar (Fankhauser und Schenkel, DE 3 810 896) ist.

Transdermale therapeutische Systeme zur Abgabe von Estradiol und/oder Gestagenen weisen jedoch die Nachteile auf, daß sie entweder Ethanol enthalten oder die potentielle Gefahr besitzen, daß der Wirkstoff im Laufe der Zeit rekrystallisiert.

Aus der DE-OS 32 05 258 und der EP 0 285 563 ist bekannt, Estradiol und Ethanol gleichzeitig in einer Pflasterfor-

mulierung zu verabreichen. Die Herstellung dieses Pflasters ist jedoch sehr aufwendig und der Tragekomfort nach Applikation aufgrund der fehlenden Flexibilität gering.

Die EP O 285 563 beschreibt ein transdermales therapeutisches System für die kombinierte Applikation von Oestrogenen und Gestagenen. Das Reservoir erhält die Wirkstoffformulierung, gegebenenfalls eine Membran sowie Ethanol als perkutanes absorptionsverbesserndes Mittel. Da die Freisetzung des Wirkstoffes hauptsächlich von der Membran gesteuert wird, unterscheidet sich dieses transdermale therapeutische System grundsätzlich von dem Wirkstoffpflaster gemäß der vorliegenden Erfindung. Der Kleber hat bei dem dort beschriebenen Pflaster lediglich die Funktion, das Pflaster auf der Haut zu befestigen. Daß er einen Beitrag zur Steuerung der Wirkstofffreisetzung zu leisten vermag, ist nicht seine Hauptaufgabe, sondern lediglich ein - möglicherweise sogar unerwünschter - Nebeneffekt. Es handelt sich hierbei um ein sogenanntes "Beutelpflaster", da sich die Wirkstoffzubereitung in einem Beutel, bestehend aus undurchlässiger Rückschicht und Membran mit Kleberschicht befindet. In Folge seines komplizierten Aufbaues ist die Herstellung des Pflasters sehr aufwendig, da die Einzelkomponenten separat hergestellt und dann in einem weiteren Arbeitsgang zu einem Pflaster zusammengefügt werden müssen.

Die EP O 275 716 beschreibt ein - im Gegensatz zu dem erfindungsgemäßen einschichtigen System - zweischichtiges transdermales System zur simultanen Verabreichung von einem oder mehreren Oestrogenen, die in der Polymerschicht gelöst oder microdispergiert sind. Die Haftschrift enthält dabei außer den Wirkstoffen Substanzen, die die transdermale Absorption verbessern. Polymer- und Haftschrift können aus Polyacrylaten, Silikonen oder Poliisobutylenen bestehen.

In der EP O 072 251 ist eine flexible, flüssigkeitsabsorbierende, medikamentöse Bandage beschrieben. Das an der

flexiblen Rückschicht befestigte Substrat besteht aus einer hydrophilen Matrix auf der Basis von hydrophilen hochmolekularen Polysacchariden und/oder Polyacrylsäure, Polyacrylamid, Ethylen-Vinylacetat-Copolymeren und anderen Polymeren sowie einer flüssigen Phase auf der Basis einer Lösung oder Emulsion aus Kohlehydrat, Proteinen und mehrwertigen Alkoholen und verschiedenen Wirkstoffen, u.a. auch Hormonen. Wesentliches Merkmal dieser Erfindung ist der feuchttigkeitsabsorbierende Kleber.

Die EP O 328 806 beschreibt ein membranfreies, transdermales therapeutisches System, dessen Matrix aus einem Polyacrylatkleber, einem Lösemittel, einem Penetrationsverbesserer und Oestrogenen, dessen Derivaten und Kombinationen davon besteht.

In der WO 87/07 138 ist ein Estradiol-Pflaster auf der Basis einer Rückschicht, einer den Wirkstoff enthaltenden Matrix und einem Haftkleber, der mit einer wiederablösbaren Schutzschicht abgedeckt ist, beschrieben. Die Herstellung von Matrix und Haftkleber erfolgen in technologisch sehr aufwendigen Arbeitsvorgängen durch Homogenisieren, Entgasen, Beschichten, Trocknen und Vereinzeln. In einer Ausführungsform muß die Rückschicht sogar mit einem Haftkleber beschichtet werden, was einen weiteren Arbeitsgang bedingt. Das Zusammenfügen der einzelnen Teile erfolgt in einem separaten Arbeitsgang. Die Herstellung des Pflasters ist also insgesamt sehr aufwendig und kompliziert.

Aus der US-PS 4 624 665 sind Systeme bekannt, die im Reservoir den Wirkstoff in mikroverkapselter Form enthalten. Das Reservoir ist eingebettet zwischen Rückschicht und einer Membran. Der äußere Rand des Systems ist mit einem Haftkleber ausgerüstet. Der Aufbau und die Herstellung dieses Systems ist sehr kompliziert, da der Wirkstoff mikroverkapselt und in einer flüssigen Phase homogen verteilt werden muß, die dann in weiteren Arbeitsgängen

zwischen Rückschicht und Membran eingebettet wird. Zusätzlich muß das System dann mit einem klebenden Rand versehen und mit einer Schutzschicht abgedeckt werden.

Es sind weiterhin aus der EP 0 186 019 Wirkstoffpflaster bekannt, bei denen einer Kautschuk-/Klebharzmasse in Wasser quellbare Polymere zugesetzt sind und aus denen Estradiol freigesetzt werden kann. Es hat sich jedoch gezeigt, daß die Freisetzung von Estradiol aus diesen Wirkstoffpflastern viel zu gering ist und nicht den therapeutischen Erfordernissen entspricht.

In der DE-OS 20 06 969 ist ein Pflaster oder ein Haftverband mit Systemwirkung beschrieben, bei dem empfängnisverhütende Substanzen in die Klebstoffkomponente oder den Klebstofffilm eingearbeitet werden. Aus dieser Schrift ist zu entnehmen, daß der Klebstoff ein Acrylat sein kann.

Die DE-OS 39 33 460 beschreibt ein oestrogenhaltiges Wirkstoffpflaster auf der Basis von Homo- und/oder Copolymeren mit mindestens einem Derivat der Acryl- oder mit Methacrylsäure in Kombination mit in Wasser quellbaren Substanzen .

Es hat sich jedoch gezeigt, daß haftklebende transdermale therapeutische Matrix-Systeme, die den Wirkstoff teilweise oder vollständig gelöst enthalten, die potentielle Gefahr beinhalten, daß der Wirkstoff im Laufe der Zeit rekristallisiert. Dadurch sinkt die Wirkstofffreisetzung und das oestrogenhaltige Pflaster entspricht nicht mehr den therapeutischen Erfordernissen.

Ein weiterer Nachteil dem Stand der Technik entsprechender Systeme ist der Einsatz von Enhancern, welcher eine grundsätzlich unerwünschte zusätzliche Beeinflussung der Haut mit dem Risiko der Irritation mit sich bringt. Weitere Nachteile liegen in dem aufwendigen Aufbau solcher Systeme

(Einsatz mehrerer wirkstoffhaltiger Schichten, Verwendung von Steuermembranen), die das fertige Produkt beim Verwender in der Regel unakzeptabel machen.

Es ist somit Aufgabe der Erfindung, die oben angeführten Nachteile zu vermeiden und ein stabiles, d.h. rekristallisationsfreies, oestrogenhaltiges Pflaster bzw. transdermales therapeutisches System zur Verfügung zu stellen, dessen Freisetzung sich durch Lagerung nicht verändert, wobei dessen Aufbau möglichst dünn zu gestalten ist und bei dessen therapeutischem Einsatz die Haut über die Wirkstoffe Estradiol und Gestagen hinaus nicht mit hautbeeinflussenden Stoffen (Enhancern) behandelt wird.

Überraschenderweise hat sich gezeigt, daß die Aufgabe dadurch gelöst wird, daß der oestrogenhaltige Haftkleber überwiegend aus Estern des Kolophoniums aufgebaut ist.

Von Vorteil ist hierbei die zusätzliche Verwendung eines Styrol-Isopren-Blockcopolymers und hydrierter Harzsäuren oder deren Derivate in der aktiven Schicht, welche beispielsweise eine therapeutisch erforderliche Menge der Wirkstoffe Estradiol und Levonorgestrel enthält.

Eine Kombination der beiden Hilfsstoffe, des Styrol-Isopren-Block-Copolymers, das als kohäsive Komponente dient, und der hydrierten Harzsäuren oder deren Derivate, die als klebrigmachende Stoffe fungieren, ergibt nicht nur einen gut klebrigen und kohäsiven Kautschukkleber, sie stellt darüberhinaus herausragende biopharmazeutische Eigenschaften bei, insbesondere gute Hautverträglichkeit und Permeationsfähigkeit, sowie eine Vermeidung der Rekristallisation der Wirkstoffe.

Die Erfindung betrifft somit ein transdermales therapeutisches System zur kontrollierten Abgabe von Estradiol oder seinen pharmazeutisch unbedenklichen Derivaten allein oder in Kombination mit Gestagenen, bestehend aus einer

Rückschicht, einem damit verbundenen wirkstoffhaltigen Reservoir, das unter Verwendung von Haftklebern hergestellt ist, und einer wiederablösbaren Schutzschicht, wobei der Haftkleber Ester des Kolophoniums und Hilfsstoffe enthält.

Zu den Estern des Kolophoniums gehören z.B. Methylester, der Glycerinester, der Pentaerythritester, der maleinsäuremodifizierte Pentaerythritester, der maleinsäuremodifizierte Glycerinester und der Triethylenglycolester. Der Anteil an Estern des Kolophoniums in dem estradiolhaltigen Haftkleber beträgt 55-92 Gew.-%, vorzugsweise 60-90 Gew.-% und ganz besonders bevorzugt 70-88 Gew.-%. Desweiteren kann der Haftkleber Ester des hydrierten Kolophoniums enthalten.

Besonders bevorzugte Ester des Kolophoniums sind der Triethylenglycolester, der Glycerinester und der Pentaerythritester des hydrierten Kolophoniums.

In einer weiteren Ausführungsform können weitere Bestandteile des estradiolhaltigen Haftklebers Polymere sein, die ausgewählt sind aus der Gruppe der Styrol-Butadien-Styrol-Blockcopolymere, Styrol-Isopren-Styrol-Blockcopolymere, Styrol-Ethylen-Butylen-Styrol-Blockcopolymere, Ethylen-Vinylacetat-Copolymere, Polyvinylpyrrolidon, Cellulosederivate und Polymere auf der Basis von Acrylsäure- und Methacrylsäurederivaten. Diese Polymere sind in einer Konzentration von 6-25 Gew.-% in der estradiolhaltigen Klebmasse enthalten.

Das rekrystallisationsfreie estradiolhaltige Pflaster enthält im Reservoir Estradiol und seine pharmazeutisch unbedenklichen Derivate allein oder in Kombination mit Gestagenen in einer Konzentration von insgesamt 2-15 Gew.-%, und zwar in einem molaren Verhältnis von 1 : 1 bis 1 : 10.

Das estradiolhaltige Reservoir kann mindestens einen Bestandteil der Gruppe enthalten, welche Alterungsschutz-

mittel, Weichmacher, Antioxidantien und Absorptionsverbesserer umfaßt. Geeignete Weichmacher sind dem Fachmann bekannt und beispielsweise in der DE 37 43 949 beschrieben. Das estradiolhaltige Reservoir enthält üblicherweise Weichmacher in einem Anteil von 0-5 Gew.-%.

Weiterhin sind im wirkstoffhaltigen Reservoir auch Alterungsschutzmittel in einer Konzentration von 0-1 Gew.-% enthalten. Diese sind dem Fachmann bekannt und Z.B. in der DE 37 43 946 beschrieben.

Das estradiolhaltige Reservoir kann sowohl aus Lösung als auch aus der Schmelze erzeugt werden.

Für den Fall, daß das Reservoir keine ausreichende Eigenklebrigkeit zur Haut aufweist, kann dieses mit einer Haftkleberschicht oder mit einem haftklebenden Rand versehen werden. Auf diese Weise ist es gewährleistet, daß das transdermale Pflaster über den gesamten Applikationszeitraum auf der Haut haftet.

Ein besonders bevorzugter Aufbau des transdermalen estradiolhaltigen Pflasters ist das Matrix-System, bei dem bekanntlich die Matrix die Steuerung für die Wirkstofffreisetzung übernimmt und diese dem Vt-Gesetz nach Higuchi gehorcht. Das bedeutet jedoch nicht, daß nicht in besonderen Fällen auch das Membran-System angezeigt ist. Hierbei ist zwischen Reservoir und Haftkleberschicht eine die Wirkstofffreisetzung steuernde Membran angebracht.

Die Dicke des transdermalen Pflasters richtet sich nach den therapeutischen Erfordernissen und kann entsprechend angepaßt werden. Sie liegt üblicherweise im Bereich von 0,03-0,4 mm.

Eine bevorzugte Einsatzform stellt weiterhin ein monolithisches Matrix-Transdermales therapeutisches System dar, das aus einer im wesentlichen wirkstoffundurchlässigen

Rückschicht, der eigentlich aktiven Matrix-Schicht (welche die erfindungsgemäßen Wirk- und Hilfsstoffe enthält) sowie einer wiederablösbaren Schutzschicht besteht.

Wie in den Beispielen gezeigt, weisen solche Systeme bei gegenüber dem Stand der Technik vereinfachtem Aufbau und geringerem Aufwand verbesserte, auch gleichmäßigere, Permeationsdaten beider Wirkstoffe auf.

Es hat sich überraschend gezeigt, daß eine solche, aus vorwiegend lipophilen sowie vergleichsweise schwach diffusiblen Polymeren und Harzen aufgebaute Rezeptur zu Human-Blutspiegeln führt, die sich mit Systemen nach dem Stand der Technik bei vergleichbar geringem Aufwand nicht erzeugen lassen.

Kautschukkleber galten bisher als weniger geeignet für die Abgabe von Estradiol an die Haut. So wird dem in EP 0 186 019 beschriebenen Gedanken, Kautschukkleber (hier unter Zusatz von wasserquellbaren Stoffen) zu verwenden, in EP 0 421 454 (S. 2, Z. 54 f.) widersprochen: eine ausreichende Freisetzung von Estradiol sei in diesen gering diffusiblen und nur schwach lösenden Polymeren nicht gegeben.

Beide nach der Erfindung wesentlichen Einsatzstoffe, Styrol-Isopren-Blockcopolymer und hydrierte Harzsäuren bzw. deren Derivate, sind seit langer Zeit als klassische Grundmaterialien von Heftpflastern erfolgreich im Einsatz und weisen gut Verträglichkeit auf. Unter der Begriff "Hydrierte Harzsäuren" werden vom Naturprodukt "Kolophonium" abgeleitete Verbindungen verstanden. Kolophonium findet als Gemisch nativer Harzsäuren vor allem in chemisch modifizierter Form breite Verwendung in Bedarfsgegenständen, Kosmetika, Lebensmittelverpackungen, Kaugummi etc.. Dabei handelt es sich um den nach Abdestillieren von Terpentinöl verbleibenden harzigen Rückstand des Rohproduktes Terpen-

tinbalsam, der von verschiedenen Kiefernarten aus überwiegend subtropisch-mediterranen Klimazonen stammt.

Das Rohprodukt ist eine spröde, harzartige, bei ca. 73-80°C erweichende Masse mit einer Dichte um 1,07 g/ml. Die Modifizierung des Kolophoniums für den Zweck der Verwendung in transdermalen Systemen dient der Stabilisierung gegen Sauerstoffeinflüsse durch Hydrierung sowie der Verbesserung der Alkalistabilität durch Veresterung. Durch Hydrierung und gegebenenfalls Derivatisierung wird das Material für den vorgesehenen Zweck geeigneter gemacht. Wichtige, für den erfindungsgemäßen Zweck verwendbare Ester sind z.B. Glycerinester, Pentaerythritester, Methylester sowie auch andere hautverträgliche Derivate des hydrierten Kolophoniums.

Synthetische Kautschukpolymere spielen eine bedeutende Rolle bei der Herstellung von transdermalen therapeutischen Systemen wie auch von Wundpflastern. Ihr Vorteil liegt in einer erheblichen Verbesserung der mechanischen Eigenschaften eines transdermalen therapeutischen Systems. In dieser Hinsicht haben sich die Styrol-Isopren-Styrol-Blockcopolymere besonders bewährt. Durch das Unterteilen der Polymerkette in einen Mittelblock aus noch beweglichen lange-kettigen Polyisopreneinheiten und die beiden Polystyrolenden als "Ankerpunkte" entsteht in der Matrix ein dreidimensionales Netzwerk, das für weitgehend konstante Geometrie auch während der Lagerung sorgt. Dabei ist es im einzelnen nicht entscheidend, welches Molekulargewicht oder welches Verhältnis zwischen dem Anteil der Styrol-domänen und der Polyisopren-domänen nun tatsächlich vorliegt. Wichtig ist vielmehr die Einstellung der korrekten Klebrigkeit und Kohäsion. Beispielsweise bewirkt ein erhöhter Harzanteil verbesserte Klebrigkeit auf der Haut, aber auch weichere Konsistenz der Matrix. In der Regel wird man mit einem Anteil von rund einem Drittel des Blockcopolymers rechnen, der nach Zusatz der Wirkstoffe verbleibende Rest sind biokompatible Harz-

derivate.

Auch wenn ein einschichtiger Aufbau des transdermalen therapeutischen Systems wegen der Einfachheit der Funktion Vorteile aufweist, ist es ohne weiteres möglich, ein solches Matrixsystem erfindungsgemäß zum Beispiel mit einer dünnen, zusätzlichen Klebschicht zur Haut hin zu versehen. Auch läßt sich zur besseren Verankerung auf der Rückschicht eine dünne Haftschrift auflaminieren. Solche Zusatzschichten können aus einem Kautschuk-Harz-Gemisch oder aber auch z.B. acrylsäure-esterhaltigen Copolymeren bestehen. Sie können auch dann verwendet werden, wenn sie vor dem Laminieren keine Beladung mit Wirkstoffen erhalten haben, da beim kurzzeitigen Zwischenlagern des Gesamtlaminates ein Diffusionsausgleich stattfindet.

Im folgenden wird die Erfindung anhand von Beispielen weiter erläutert.

Beispiel 1:

73,1 g Triethylenglycolester von hydriertem Kolophonium
(Staybelite Ester 3E/Fa. Hercules) und
9,8 g Glycerinester von hydriertem Kolophonium
(Staybelite Ester 10E/Fa Hercules)

werden bei 100°C 5 Minuten durch Kneten gemischt. Anschließend werden 2,5 g Estradiol zugegeben. Es wird 30 Minuten geknetet. Nach Aufheizen auf 140°C werden 14,6 g Ethylcellulose N50NF (Fa. Hercules) portionsweise zugegeben und anschließend noch 2,5 St. geknetet.

Die so erhaltene wirkstoffhaltige Klebmasse wird mit einer Hotmelt-Beschichtungsanlage (Düsenauftragssystem) so auf eine wiederablösbare Schutzschicht (Hostaphan RN 100 einseitig mit Silikon beschichtet - Fa. Kalle) beschichtet, daß ein wirkstoffhaltiges Reservoir mit

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einem Flächengewicht von 80 g/m^2 resultiert. Auf dieses Reservoir wird die undurchlässige Rückschicht (Polyesterfolie, $15 \mu\text{m}$ dick) aufkaschiert. Anschließend werden 16 cm^2 -große Wirkstoffpflaster gestanzt.

Beispiel 2:

Die Herstellung erfolgt wie unter Beispiel 1 beschrieben, wobei der Weichmacher mit den beiden Staybelite Estern 3E und 10E gemeinsam geknetet wird.

Beispiele 3-9:

Die Herstellung erfolgt wie unter Beispiel 1 beschrieben, jedoch mit den in Tabelle 1 (Herstellformel) angegebenen Rohstoffen und Mengen.

Analytik:

Die Wirkstofffreisetzung der 16 cm^2 -großen transdermalen Pflaster wird nach der in der USP XXII beschriebenen Rotating bottle-Methode in 0,9%iger Kochsalzlösung bei 37°C bestimmt.

Zur Messung der Mäusehautpenetration wird die Haut von haarlosen Mäusen in die Franz-Zelle eingespannt. Auf die Haut wird ein oestradiolhaltiges Pflaster mit einer Fläche von $2,54 \text{ cm}^2$ aufgeklebt und die Wirkstofffreisetzung bei 37°C (Akzeptormedium: 0,9%ige Kochsalzlösung) gemessen (Literatur: Umesh V. Banakar Pharmaceutical dissolution testing (1. Auflage - 1991)).

Die Prüfung auf Rekristallisationserscheinung wird visuell im Gegenlicht durchgeführt.

Die Ergebnisse sind in Tabelle 2 dargestellt.

Tabelle 1: Herstellformel (Angaben in g)

Bsp.	Ethylcellulose N50NF	Staybelite Ester		Weichmacher Miglyol 812	Estradiol	Anti- oxidantien
		3E	10E			
1	14,6	73,1	9,8	---	2,5	
2	14,3	71,6	9,6	2,0	2,5	
3	10,1	75,4	10,0	2,0	2,5	
4	7,7	77,5	10,3	2,0	2,5	
5	14,3	71,6	9,5	2,0	2,5	0,1 BHT
6	14,3	71,6	9,5	2,0	2,5	0,1 BHA
7	14,3	71,6	9,5	2,0	2,5	0,1 BHT:BHA =1:1
8	14,3	71,6	9,6	2,0 Isopropyl- palmitat	2,5	
9	14,3	71,6	9,5 ⊗	2,0	2,5	

BHT = Butylhydroxitoluol

BHA = Butylhydroxianisol

⊗ = Foral 105 (Pentaerythritester von hydriertem Kolophonium)

Tabelle 2: Analysenergebnisse

Bsp.	Estradiolgehalt $\mu\text{g}/16\text{cm}^2$	in vitro-Freisetzung $\mu\text{g}/16\text{cm}^2 \cdot 24 \text{ Std.}$	Mäusehautpenetration $\mu\text{g}/16\text{cm}^2 \cdot 24 \text{ Std.}$	Rekristallisation
1	3200	614	225	keine
2	3200	1240	300	"
3	3200	722	235	"
4	3200	713	268	"
5	3200	624	228	"
6	3200	624	249	"
7	3200	620	205	"
8	3200	686	232	"
9				
nach DE 3933460	3200	2400	125	erheblich

Wie die Tabelle zeigt, erhält man eine deutlich bessere Penetration durch die Mäusehaut wie das Vergleichsbeispiel unter DE 3933460 unter Beweis stellt. Parallel dazu kann festgestellt werden, daß die Rekristallisation der erfindungsgemäßen Beispiele völlig unterbleibt.

Beispiel 10:

1,0 g 17- β -Estradiol
1,3 g Levonorgestrel
60,0 g Cariflex^R TR 1107 (Styrol-Isopren-Styrol-Block-
copolymer) 138,0 g Foral^R 85 (thermoplastisches Ester-
harz aus Kolophoniumderivaten)
200,0 g Benzin (Siedebereich 80-100°C)

werden bei Raumtemperatur in einem zylindrischen Glas-
gefäß bis zum Erreichen einer gleichmäßigen Suspension
gerührt und anschließend auf einer kontinuierlich
arbeitenden Beschichtungsanlage auf eine 100 Mikrometer
dicke, siliconisierte Polyesterfolie so beschichtet,
daß eine Schichtdicke von 110 g/m² (bezogen auf den
lösemittelfreien Anteil) resultiert. Der Ausstrich wird
jeweils 3 Minuten lang bei 40 Grad C, 60 Grad C, 75 Grad
C und bei 125 Grad C getrocknet. Sofort wird eine 12
Mikrometer dicke Polyesterfolie luftblasenfrei unter
Walzendruck auf die getrocknete Schicht aufgelegt (zu-
kaschiert). Durch Stanzung mit Henkellocheisen werden
transdermale Systeme von 20 cm² erhalten.

Beispiel 11: Herstellung eines erfindungsgemäßen Systems

1,5 g 17- β -Estradiol
1,5 g Levonorgestrel
70,0 g Styrol-Isopren-Styrol-Blockcopolymer
150,0 g thermoplastisches Esterharz aus Kolophonium-
derivaten

werden in einem beheizbaren Kneter bei 150°C unter Stick-
stoff innerhalb von 24h verschmolzen und knetend ver-
einigt. Auf einer kontinuierlich arbeitenden Beschich-
tungsanlage wird in einer Schichtdicke von 100 Mikro-
meter eine 19 μ m dicke Polyesterfolie mit der Schmelze
beschichtet. Dies kann bei 140°C in einem Schmelzauf-
tragswerk oder auch bei ca. 80-100°C mittels eines
Extruders geschehen. Anschließend wird eine 150 Mikro-

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meter dicke, siliconisierte Polyesterfolie, vorbe-
schichtet mit 20 g/m^2 eines Acrylestercopolymers
(Durotak^R 280-2516), luftblasenfrei unter Walzendruck
auf die getrocknete Schicht aufgelegt (zukaschiert).
Durch Stanzung mit Henkellocheisen werden transdermale
Systeme von 20 cm^2 erhalten.

P A T E N T A N S P R Ü C H E

1. Wirkstoffhaltiges transdermales therapeutisches System zur kontrollierten Abgabe von Estradiol oder seinen pharmazeutisch unbedenklichen Derivaten allein oder in Kombination mit Gestagenen aus einer Rückschicht, einem damit verbundenen wirkstoffhaltigen Reservoir, das unter Verwendung von Haftklebern hergestellt ist, und einer wiederablösbaren Schutzschicht, dadurch gekennzeichnet, daß der Haftkleber Ester des Kolophoniums enthält.

2. Transdermales therapeutisches System nach Anspruch 1, enthaltend die Wirkstoffe Estradiol und Levonorgestrel, dadurch gekennzeichnet, daß die aktive Schicht des Systems neben den Wirkstoffen ein Styrol-Isopren-Blockcopolymer und hydrierte Harzsäuren oder deren Derivaten enthält.

3. Transdermales therapeutisches System nach Anspruch 1, dadurch gekennzeichnet, daß der Haftkleber Ester des Kolophoniums in einem Anteil von 55-92 Gew.-% enthält.

4. Transdermales therapeutisches System nach Anspruch 1, dadurch gekennzeichnet, daß der Haftkleber Ester des Kolophoniums in einem Anteil von 60-90 Gew.-% enthält.

5. Transdermales therapeutisches System nach Anspruch 1, dadurch gekennzeichnet, daß der Haftkleber Ester des Kolophoniums in einem Anteil von 70-88 Gew.-% enthält.

6. Transdermales therapeutisches System nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß Ester des Kolophoniums aus der Gruppe bestehend aus Methyl-ester, Glycerinester, Pentaerythritester, maleinsäuremodifiziertem Pentaerythritester, maleinsäuremodifiziertem Glycerinester und Triethylenglycolester, ausgewählt sind.

7. Transdermales therapeutisches System nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die Konzentration an Estradiol in der aktiven Schicht zwischen 0,2 und 2 Gewichtsanteilen, bevorzugt zwischen 0,7 und 1,4 Gewichtsanteilen liegt.

8. Transdermales therapeutisches System nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die Konzentration an Levonorgestrel in der aktiven Schicht zwischen 0,1 und 1,6 Gewichtsanteilen liegt.

9. Transdermales therapeutisches System nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß die Schichtdicke der aktiven Schicht zwischen 30 und 300 μm , bevorzugt zwischen 70 und 120 μm liegt.

10. Transdermales therapeutisches System nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß der Anteil an Styrol-Isopren-Blockcopolymer in der aktiven Schicht 10 bis 45 Gewichtsprozent, vorzugsweise 15 bis 33 Gewichtsprozent beträgt.

11. Transdermales therapeutisches System nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß es einen oder beide der Kombinationspartner Levonorgestrel oder Estradiol teilweise in Suspension enthält.

12. Transdermales therapeutisches System nach einem oder mehreren der vorstehenden Ansprüche, dadurch gekennzeichnet, daß sich Estradiol teilweise in Form von Estradiol-Kristallen im transdermalen therapeutischen System befindet, wobei Estradiol-Kristalle im wesentlichen aus gefälltem Estradiol-Anhydrat bestehen.

13. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 12, dadurch gekennzeichnet, daß der Haft-

kleber Ester des hydrierten Kolophoniums enthält.

14. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 13, dadurch gekennzeichnet, daß der Haftkleber Polymere enthält.

15. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 14, dadurch gekennzeichnet, daß der Haftkleber Polymere in einer Konzentration von 6-25 Gew.-% enthält und diese ausgewählt sind aus der Gruppe der Styrol-Butadien-Styrol-Blockcopolymere, Styrol-Isopren-Styrol-Blockcopolymere, Styrol-Ethylen-Butylen-Styrol-Blockcopolymere, Ethylen-Vinylacetat-Copolymere, Polyvinylpyrrolidon und Cellulosederivate und Polymere auf der Basis von Acrylsäure- und Methacrylsäurederivaten.

16. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 15, dadurch gekennzeichnet, daß das Reservoir Estradiol oder seine pharmazeutisch unbedenklichen Derivate allein oder in Kombination mit Gestagenen in einer Konzentration von insgesamt 2-15 Gew.-%, und zwar in einem molaren Verhältnis von 1 : 1 bis 1 : 10 enthält.

17. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 16, dadurch gekennzeichnet, daß das Reservoir mindestens einen Bestandteil aus der Gruppe, bestehend aus Alterungsschutzmitteln, Weichmachern, Antioxidantien und Absorptionsverbesserern, enthält, wobei der Weichmacher in einer Konzentration von 0-5 Gew.-% und das Alterungsschutzmittel in einer Konzentration von 0,1 Gew.-% enthalten ist.

18. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 17, dadurch gekennzeichnet, daß der Haftkleber ein Lösemittelhaftkleber ist.

19. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 18, dadurch gekennzeichnet, daß der Haftkleber ein Schmelzhaftkleber ist.

20. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 19, dadurch gekennzeichnet, daß das Reservoir aus mehreren Schichten besteht.

21. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 20, dadurch gekennzeichnet, daß das Reservoir mit einer zusätzlichen haftklebenden Schicht bzw. mit einem haftklebenden Rand versehen ist.

22. Transdermales therapeutisches System nach einem oder mehreren der Ansprüche 1 bis 21, dadurch gekennzeichnet, daß zwischen Reservoir und Haftklebeschicht eine die Wirkstofffreisetzung steuernde Membran angebracht ist.

23. Verfahren zur Herstellung eines transdermalen therapeutischen Systems nach einem der vorstehenden Ansprüche, dadurch gekennzeichnet, daß es die folgenden Schritte umfaßt: Kneten der Mischung aus Estern des Kolophoniums bei erhöhter Temperatur bis zur Homogenisierung, Einarbeitung von Wirkstoff(en) und mindestens einem Polymer bei Lösetemperatur, nach Homogenisierung Beschichten einer wiederablösbaren Schutzschicht mit der wirkstoffhaltigen Klebmasse und Aufkaschieren der Rückschicht.

24. Verwendung des wirkstoffhaltigen Pflasters nach einem der Ansprüche 1 bis 22 für therapeutische Zwecke in der Human- und Veterinärmedizin.

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/EP 94/01279A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K9/70 A61K31/565

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE,C,42 23 360 (LTS LOHMANN THERAPIE-SYSTEME GMBH & CO KG) 8 April 1993	1,3,4, 6-24
Y	see the whole document see column 6; examples 2,3 ---	2
Y	EP,A,0 285 563 (CIBA-GEIGY AG) 5 October 1988 cited in the application see claim 1 ---	2
T	EP,A,0 607 434 (HISAMITSU PHARMACEUTICAL CO.) 27 July 1994 see page 4, line 16 - line 30 see page 8; example 13	1-24
A	& WO,A,93 04677 (HISAMITSU PHARMACEUTICAL CO.) 18 March 1993 -----	1-24

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/01279

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INTERNATIONALER RECHERCHENBERICHT

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PCT/EP 94/01279

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
 IPK 5 A61K9/70 A61K31/565

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C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	DE,C,42 23 360 (LTS LOHMANN THERAPIE-SYSTEME GMBH & CO KG) 8. April 1993	1,3,4, 6-24
Y	siehe das ganze Dokument siehe Spalte 6; Beispiele 2,3 ---	2
Y	EP,A,0 285 563 (CIBA-GEIGY AG) 5. Oktober 1988 in der Anmeldung erwähnt siehe Anspruch 1 ---	2
T	EP,A,0 607 434 (HISAMITSU PHARMACEUTICAL CO.) 27. Juli 1994 siehe Seite 4, Zeile 16 - Zeile 30 siehe Seite 8; Beispiel 13	1-24
A	& WO,A,93 04677 (HISAMITSU PHARMACEUTICAL CO.) 18. März 1993 -----	1-24

☐ Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

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Datum des Abschlusses der internationalen Recherche

26. August 1994

Absenddatum des internationalen Recherchenberichts

02. 09. 94

Name und Postanschrift der Internationalen Recherchenbehörde
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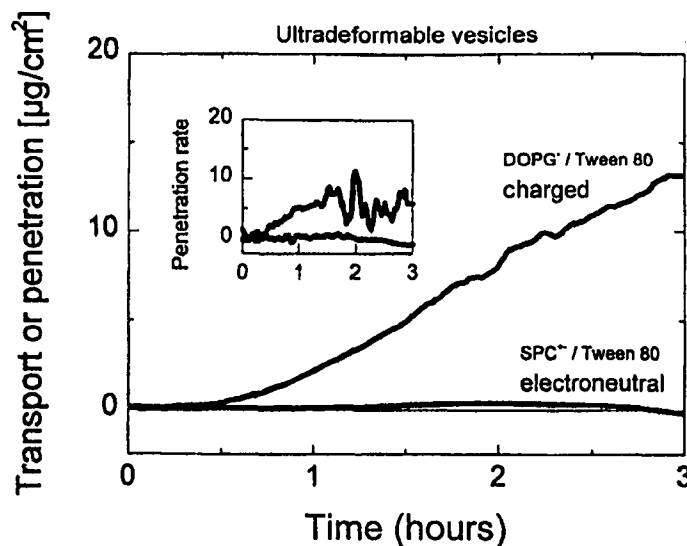
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(54) Title: ELECTRICALLY CONTROLLED TRANSPORT OF CHARGED PENETRANTS ACROSS BARRIERS

(57) Abstract

It is an object of the invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and/or said agents being provided with sufficient electrical charges, to enable and/or

control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. It is another object of the invention to provide a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier.



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Electrically controlled transport of charged penetrants across barriers

5 Field of the invention

This invention relates to a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than
10 the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to
15 enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability. This invention also relates to a method for effecting the electrically driven transport of said penetrants and associated molecules through the
20 pores in a barrier.

Background of the invention

25 Charged entities may migrate spontaneously from the high to the low electrostatic potential site, unless prevented from doing so by an obstacle, such as a barrier. The driving electrostatic force is proportional to the total charge on an entity and to the electrostatic potential difference. Material flow also depends on the system's resistance to resulting motion. Consequently, the electrically driven transport across a barrier is
30 sensitive to the number, width and characteristics of pores in a barrier, which together define the barrier permeability, P , and its inverse, the barrier resistance. One example

for such pourous barrier is the skin, which typically contains pores (in the unwidened state) with the diameter of a few Ångstroms, approximately.

Any transcutaneous electric potential that drives an ion flux across the skin tends to widen some hydrophilic channels in the organ. This typically happens at the worst
5 packed sites between the cells, where the biggest opportunity for the transport enhancement resides.

Skin penetration by means of suitable carriers achieves a similar goal without the need to use gadgets or external sources of energy (Schätzlein, A.; Cevc, G. (1998): Non-uniform cellular packing of the stratum corneum and permeability barrier
10 function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br. J. Dermatol. 138: 583-592). The hydrophilic passages (pores) through the skin before the treatment will only let small, e.g. water, molecules pass. Such pores can be opened into wider channels, however, by the addition of sufficiently potent penetrants.

15 Electrical potential difference attempts to drive charged penetrants across the barrier; for example, across the skin (*cutis*); furthermore, an electrical permeation enhancement widens at least some hydrophilic channels in the organ. This happens nearly exclusively in the horny layer of the skin (the *stratum corneum*), which contributes most
20 to the skin permeability barrier. Depending on the size of newly opened transcutaneous pathways, it is customary to speak about electrophoresis (=iontophoresis) or electroporation, for the electrically induced flow through narrow passages and for the widening of more extended passages, respectively.

25 For example, an electric current of approximately 0.4 mA cm^{-2} or less will activate a small proportion of narrow ($\sim 0.5 \text{ nm}$) hydrophilic channels pre-existing between the cells in the skin. Such channels then remain open for many hours (Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7), but remain narrow (< 3
30 nm, in nude mice), when a transcutaneous voltage remains in the physiologically tolerable range ($< 3 \text{ V}$ for a 1 cm^2 patch).

- The widest channels are negative inside. Neutral channels are only half as wide and the positive ones are twice smaller (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. *Pharm. Res.* 7: 213-21). To date, the widest channels inferred to occur during a low-voltage electromotion through the skin were reported to be merely 20 nm in diameter or less (Aguillelle, V.; Kontturi, K.; Murtomaeki, L.; Ramirez, P. (1994) Estimation of the pore size and charge density in human cadaver skin. *J. Contr. Rel.* 32: 249-257).
- 10 The standard electrical skin permeability enhancement method (electrophoresis) therefor only can improve the transport of relatively small (<2 nm) charged molecules across the organ. Electrophoresis across the skin, consequently, is feasible for certain polypeptides but is practically useless for the delivery of proteins or other large penetrants (for reviews see refs. Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm. Res. Sep*; 8: 1121-7; Green, P. G.; Flanagan, M.; Shroot, B.; Guy, R. (1993) Iontophoretic drug delivery. In: *Pharmaceutical Skin Penetration Enhancement* (Walters, K. and Hadgraft, J., eds.) Marcel Dekker, New York, 297-319; Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. *J. Pharm. Sci.* 82: 240-3; Singh, S.; Singh, J. (1993) Transdermal drug delivery by passive diffusion and iontophoresis: a review. *Med. Res. Rev.* 13: 569-621; Singh, J.; Bhatia, K.S. (1996) Topical iontophoretic drug delivery: pathways, principles, factors, and skin irritation. *Med. Res. Rev.* 16: 285-96).
- 25 Transcutaneous channels opened by the low voltages and tolerably small currents only cover some 0.005 % of the total treated area, despite their seemingly high number ($< 3 \times 10^8 \text{ cm}^{-2}$) (Pikal, M.J. (1990) Transport mechanisms in iontophoresis. I. A theoretical model for the effect of electroosmotic flow on flux enhancement in transdermal iontophoresis. *Pharm. Res.* 7: 118-26). More extended local skin perforations, which are created by a higher voltage (> 150 V), are rarer, but normally
- 30

persist in the skin for several days in the form of lesions.

Transcutaneous channels size is affected by a number of parameters. For example, a channel widens with increasing electrostatic potential as well as with decreasing supporting electrolyte concentration, but the latter variation is practically only possible within relatively narrow limits. Moreover, no teaching was given to date on how to improve the transport across a barrier by using such principles. Perhaps, this is due to the fact that the electrophoretic channels in the skin are charge and molecular weight selective (Banga, A.K.; Chien, Y.W. (1993) Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. Pharm. Res. 10: 697-702) but not very sensitive to the agent lipophilicity variation (Green, P.G.; Hinz, R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. Pharm. Res. Sep; 8: 1121-7).

Repeated electrophoretic delivery through the same skin area results in a divergent, but typically greater, flux across the barrier which makes data interpretation and recommendations difficult (Heith, M.C.; Williams, P.L.; Jayes, F.L.; Chang, S.K.; Riviere, J.E. (1993) Transdermal iontophoretic peptide delivery: in vitro and in vivo. Studies with luteinizing hormone releasing hormone. J. Pharm. Sci. 82: 240-3). Further complications arise from the electrical current through the appendages in the skin, such as hair follicles.

Electrical opening of channels through the skin is reflected in the following contribution to the skin permeability,

$$P_{i,el} = \text{Skin Permeability to Ions} = (c_i Z_i F / RT) D_i / d_s$$

which needs to be added to the permeability observed with no electrical force applied across the barrier. In addition to the pores opening, a transcutaneous electrical potential gradient ($\Delta\psi_{el}$) also activates the electromotive forces which try to drive charged penetrants through the channels. This gives rise to an additional term in Fick's transport

equation used to model transbarrier (e.g. transcutaneous) transport (see further discussion):

$$j_i = \dots a c_i + P_{i,el} \Delta \psi_{el}$$

5

c_i is the bulk concentration of substance i , $Z_i F$ is its molar charge (valency times Faraday constant), and RT is the molar thermal energy. D_i is the diffusivity of substance i and d , the skin thickness. Electric current corresponding to i is given by the product of i -flux and $Z_i F$, but the total current comprises all individual contributions and thus is given by the sum of such contributions.

10

Electrophoresis represents the direct flow of charged molecules in an electric field under the electrode. Drug molecules must therefore be placed at electrodes having a polarity of the same charge as the agent. Under such circumstances, the flux magnitude is proportional to the net number of charges on each migrating molecule and to the applied potential. Further important factors are drug concentration and diffusivity in the barrier or skin (see equation given later in the text).

15

Owing to absolute differences in concentrations, electrophoretic current normally also comprises contributions from the supporting electrolyte ions (e.g. Na^+ , Cl^-). These are often dominant making the drug contribution only a minor part of the measured current. Increased ion concentration under the electrodes therefore lowers the useful part of electrophoretic flow (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Pikal, M.J.; Shah, S. (1990c) Transport mechanisms in iontophoresis. III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes. Pharm. Res. 7: 222-9), as can be seen from simple differential calculation.

25

It is therefore state of the art knowledge that physical limitations restrict maximum achievable, or tolerable, electrophoretic current across the skin: currents flowing

30

through the already opened pores dissipate electric energy; this prevents a greater increase in the channel number and size, as stated above, and minimizes the achievable transport gain. Further pore opening is also restricted by the adverse side effects of energy dissipation in the skin (skin itching and etching).

5

- Electro-osmotic flux, that is, the flow of water associated with the transported ions carrying uncharged species, also relies on hydrophilic channels in the skin and on the applied potential. Flux under an anode typically exceeds the cathodal values, probably due to the different average size of positively and negatively charged channels. The average flux value reaches a steady-state during 10 hours of constant-current iontophoresis (0.36 mA cm^{-2}) at the level a few times higher than at the beginning ($< 3 \mu\text{L h}^{-1} \text{ cm}^{-2}$; Kim, A.; Green, P.G.; Rao, G.; Guy, R.H. (1993) Convective solvent flow across the skin during iontophoresis. Pharm. Res. 10: 1315-20).
- 15 Electrically induced changes in the skin are the greatest over the first hour of electrophoresis (Pikal, M.J.; Shah, S. (1990b) Transport mechanisms in iontophoresis. II. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7: 213-21; Craane van-Hinsberg, W.H.; Bax, L.; Flinterman, N.H.; Verhoef, J.; Junginger, H.E.; Bodde, H.E. (1994) Iontophoresis of a model peptide across human skin in vitro: effects of iontophoresis protocol, pH, and ionic strength on peptide flux and skin impedance. Pharm. Res. Sep; 11: 1296-300). During this period of time, the resistance drops from $> 20 \text{ k}\Omega \text{ cm}^{-2}$ to approximately 10 % of the starting value.
- 25 Skin pretreatment with ethanol reduces (Brand, R.M.; Iversen, P.L. (1996) Iontophoretic delivery of a telomeric oligonucleotide. Pharm. Res. 13: 851-4) or else increase (Srinivasan, V.; Higuchi, W.I.; Sims, S.M.; Ghanem, A.H.; Behl, C.R. (1989) Transdermal iontophoretic drug delivery: mechanistic analysis and application to polypeptide delivery. J. Pharm. Sci. 78: 370-5) the electrophoretic transport across the organ. Most chemical skin permeation enhancers improve the electroconductivity of the skin, and thus also the electrically driven transcutaneous transport (Green, P.G.; Hinz,
- 30

R.S.; Kim, A.; Szoka, F.C. Jr.; Guy, R.H. (1991) Iontophoretic delivery of a series of tripeptides across the skin in vitro. *Pharm. Res. Sep*; 8: 1121-7); so does the pH adjustment, particularly lowering of pH. Part of this effect is due to electrophoresis and part to electro-osmosis, but increases are generally relatively small.

5

- Electrophoretic enhancement of molecular motion across the skin was only partly successful to date (for a recent survey of products and developments see: Cevc, G. (1997) *Drug Delivery Across the Skin. Exp. Opin. Invest. Drugs* 6: 1887-1937). Particularly poor results were achieved with macromolecules (see, e.g. the reviews
- 10 by Siddiqui, O.; Chien, Y. W. *Nonparenteral administration of peptide and protein drugs. Crit. Rev. Therap. Drug Carrier Syst.* 1987, 3: 195-208 and Banga, A.K.; Chien, Y.W. (1993) *Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. Pharm. Res.* 10: 697-702). For insulin, for example, the effectiveness of iontophoretic transport in the best case was
- 15 4% per hour and normally lower than 3 % per hour; part of the observed transport effect probably being due to the skin damage (Siddiqui, O.; Chien, Y. W. *Nonparenteral administration of peptide and protein drugs. Crit. Rev. Therap. Drug Carrier Syst.* 1987, 3: 195-208).
- 20 This problem is partly due to the high mass, but also to the hydrophilicity of most large molecules, which both pose tremendous difficulties to the general use of conventional skin permeation enhancement technology.
- The situation with other large penetrants is comparably bad. To date, only one
- 25 publication tackled the problem of driving large lipid aggregates, liposomes, across the skin, without finding a solution (see further discussion).
- It is therefore fair to say that no procedure was known before this invention which would ensure an efficient electromotion of large penetrants across the microporous
- 30 barriers, such as mammalian skin. Moreover, no generally applicable method was proposed to date for the opening of large pores in the skin. This is unfortunate in light

of the desire to deliver transcutaneously large molecules, such as peptides and proteins, but also due to the long standing desire to control aggregate motion across any kind of transport barrier.

- 5 The extent and mechanism of aggregate motion across biological barriers, such as the stratum corneum, is strongly disputed (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388), as is the penetration pathway through the skin. We have repeatedly discussed hydrotaxis as most important cause for the
- 10 transport of superficially hydrophilic, highly deformable vesicles through the biological barriers, such as the skin (Cevc, G. (1996) Lipid Suspensions on the Skin. Permeation Enhancement, Vesicle Penetration and Transdermal Drug Delivery. Crit. Rev. Therap. Drug Carrier Systems. 13: 257-388; Cevc, G. (1997) Drug Delivery Across the Skin. Exp. Opin. Invest. Drugs 6: 1887-1937). We argued that diffusion is not a good basis
- 15 for transporting large aggregates, i.e. lipid vesicles, accross such barriers.

The first reason for this is the very low permeability (P_a) of any big aggregate with a large effective mass, which is typically proportional to the aggregation number (n_a). Since the P_a -value correlates with the diffusion constant (D_a), the permeability and the

20 flux of such a large body both decrease linearly with the growing aggregate size. (P_a is thus proportional to $D_a \sim D_1/n_a$, where D_1 is the diffusion constant of a monomer.)

The second cause for the insignificance of aggregate diffusion is the smallness of largest achievable aggregate concentration difference across the barrier ($\Delta c_a = \Delta c_1 / n_a$, where c_1

25 is the saturated monomer concentration). As can be calculated from the first Fick's law, the flux $j_m = P_a \Delta c_a$, is hence proportional to $D_a \Delta c_a \sim D_1 \Delta c_1 / n_a^2$.

Both above mentioned phenomena, which result in $D_a(n_a \gg 1) \rightarrow 0$ and $\Delta c_a(n_a \gg 1) \rightarrow 0$, contribute to negligibly small barrier permeability for the vesicle

30 transport: $P_a(n_a \gg 1) \rightarrow 0$.

The use of water activity gradient (Δa_w), i.e. hydrotaxis, to drive transbarrier transport solves the first part of permeability problem. Our interpretation of this is the following: the aggregate independent water activity gradient exerts a similar attraction on all polar molecules in the aggregate; this strengthens proportionally the pressure acting on each aggregate, $\Delta p_{\text{hyd},a} \sim \Delta a_w RT n_a$ or on the corresponding force that drives the aggregate transport across the barrier, F_{hyd} . Both are much bigger for aggregates than for a single molecule. This compensates the smallness of aggregate concentration difference, as can be seen from the generalized Fick's equation:

10

$$j_a = P_a \Delta c_a + P_a'' \Delta a_w RT n_a \\ \sim P_a' n_a F_{\text{hyd},l}$$

$F_{\text{hyd},l}$ denotes the force acting on each monomer in the aggregate and RT is the thermal energy.

15

In order to profit maximally from an 'external' transport driving force, which is (permeant/penetrant) concentration independent, one can use easily deformable aggregates described in PCT/EP91/01596. This minimizes the increase of transport resistance with increasing aggregate number, by pushing it below the suggested linear dependence (see figure 1).

20

One example for this are the vesicles with a membrane sufficiently flexible to result in a low vesicle deformation energy. This is especially true when the vesicles are subject to strongly anisotropic (ideally: unidirectional) stress, 'force' or pressure. The combination of molecular aggregation and membrane flexibility under the corresponding conditions, therefore, may lead to vesicle motion through a barrier even when the pores in such barrier are smaller than the vesicle diameter. Significant material flow in the desired direction can result from this.

30

The same consideration applies to every external, and therefore concentration

independent, force (F_{ext}) or pressure (Δp_{ext}). The proviso for this is that aggregate-size dependent increase of the force - or of the resulting trans-barrier pressure difference - exceeds the decrease of permeability (P_a) with growing aggregate size. A net trans-barrier transport results from this. The relation is schematically illustrated in figure 1.

- 5 If the driving force increases linearly with the number of charges on each moving entity, and provided that the transport resistance of such entity is increases less rapidly than the driving force (full line), a net transport will ultimately result. Such is the situation with the penetrants which are adaptable in shape to the pores. When the penetrants get big enough, and the driving force exceeds the transport resistance, an effective transport
- 10 sets in. If the penetrants are not adaptable to the pores in a barrier, however, the barrier resistance inevitably exceeds the driving force, when the average 'penetrant' size exceeds the average diameter of a pore (dotted line).

The above reasoning applies as long as the transport driving force is constant or as long

15 as the increase of transport resistance does not exceed the size dependent ascent of driving force. Highly deformable aggregates subject to a sufficiently high external pressure ($\Delta p_{ext,a}$) provide an example for this; the opposite situation is encountered with conventional, less deformable aggregates under a similar pressure, since these aggregates will rather break at than pass through the barrier.

20

In the case of an external electrical force (F_{el}), which could lead to so-called electrophoresis, similar basic principles apply, but are more complicated and not a priori recognizable..

- 25 In the simplest, hypothetical case, in which an aggregate comprises n_a charged molecules with a charge Ze_0 each, and the corresponding counterions are the only other charged entities in the system, the rate of aggregate transport increases linearly with aggregate size or number, as well as with the electric gradient across the barrier (E). This is true as long as transport resistance remains constant, as the driving force is then
- 30 given by $F_{el} = n_a Ze_0 E$, and the P_a -value is taken to be constant. However, if the transport resistance depends on aggregate-size, and also increases with the value of n_a ,

the P_a -value decreases and can abolish the transport-rate sensitivity to the changes in aggregation number. When P_a -value decreases faster than linearly, since the transport resistance increase is more than linear, the transport rate decreases with increasing n_a -value even.

5

One would therefore only expect an efficient electrophoresis in the first of the three above mentioned cases. In reality many more problems arise. Nearly all suspensions of charged aggregates contain small ions of similar charge as aggregates, in addition to ionic aggregate components. These small, additional charges react to an electric field like aggregates and trespass the barrier in the same direction. As a consequence of this, a parasitic stream of 'small charges' begins to flow, which ultimately may dissipate the electrical potential over the barrier. If the transport resistance of such 'opportunistic charges' is smaller than that of the useful aggregates, which is usually the case, the aggregate transport can stop eventually.

15

A further expected complication in electrophoresis of large objects is the possibility that an applied electrical potential tends preferentially to pull individual charged molecules from an aggregate over the barrier, instead of transporting the whole aggregate. The expected size-dependence of transport resistance nourishes this notion, especially for the relatively strongly water soluble, charged aggregate components. The highly deformable aggregates, which consist of substances of different solubility (according to PCT/EP91/01596), fulfill such requirement. This rises the doubt about the suitability of the corresponding ultradeformable vesicles for electrophoresis.

25 The only group which published results on electrically driven material transport across the skin by using liposomes as "permeation enhancers" produced very sobering data, indeed.

In the first publication on the combined use of liposomes and iontophoresis for transdermal delivery, which should form closest prior art to the present invention, Vutla et. al. (Vutla, N.B.; Betageri, G.V.; Banga, A.K. (1996) Transdermal iontophoretic

30

delivery of enkephalin formulated in liposomes. J. Pharm. Sci., 85: 5-8) reported the following.

5 Liposomes comprised dimyristoylphosphatidylcholine /cholesterol 2/1 mol/mol mixture with an unspecified amount of cationic stearylamine or anionic phosphatidylserine, when appropriate, to make the vesicles charged. They were prepared fresh by extrusion and had a size of 110 nm. The release was much higher from neutral and negative liposomes than from the positive vesicles.

10 A current of 0.5 mA cm^{-2} density co-transported [Leu5]enkephalin, (spiked with [3H]enkephalin) across the skin from anode or cathode, depending on the charge on the molecule.

15 After 12 h of iontophoresis, liposome derived material was found in the skin at the level of approx. 2.5%, 0.75%, and 1.5% for the positive, negative and neutral vesicles, respectively; in the absence of electrical current 0.8% of material from neutral liposomes was found in the skin. No liposome derived material was recovered from receiver fluid.

20 The use of negatively charged vesicles did not enhance enkephalin delivery across the skin. The positive liposomes even reduced the delivery compared to control.

25 The polypeptide delivery into the skin was the highest (4.2%) for the neutral vesicles used in conjunction with electrophoresis (sic!), followed by the same kind of vesicles used in the absence of electrical current (passive delivery: 2.7%); anionic and cationic vesicles used with iontophoresis mediated much lower intracutaneous drug delivery of 0.5% and 0.7%, respectively.

30 The work of Vutla thus clearly shows that conventional liposomes, whether charged or uncharged, are poor mediators of the electrically driven material transport into the skin.

It was therefore not expected to date that electrical driving force could be used as a replacement of hydrotaxis for the purpose of transporting large lipid aggregates (e.g. vesicles) or other big entities across the skin.

- 5 In view of the foregoing it is therefore an object of the present invention to provide a preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being
- 10 capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores.

- It is moreover an object of the present invention to provide a method for effecting the electrically driven transport of penetrants and associated molecules through the pores in
- 15 a barrier by applying an electrical potential across the barrier.

These objects are attained by the invention as defined in the attached independent claims.

- 20 Further advantageous embodiments of the present invention are provided by appended subclaims.

Description of the invention

- 25 We found, unexpectedly, that charged ultradeformable lipid aggregates in vesicular form can be forced to cross artificial as well as natural nano-porous barriers with an externally applied electrical potential difference. The proviso for this is sufficient stress on the vesicles, which must be big enough to deform the vesicles. Such a situation is only realized with the vesicles with very flexible membranes. The process involving the
- 30 deformation of entire aggregate, the relative magnitude of vesicle adaptation to the pore

penetration is affected by the average vesicle/pore size ratio.

We also found out that the electromotion of charged aggregates is sensitive to the bulk electrolyte concentration. Unexpectedly, the measured dependence was seen to deviate, 5 qualitatively as well as quantitatively, from that expected on the basis of known electromotion of non-deformable, small penetrants across a barrier. The electrically driven motion of the highly deformable vesicles across "confining" pores therefore differs from conventional electrophoresis and provides new means for the delivery of drugs across various, including biological, barriers.

10

It stands to reason that nonocclusive pretreatment of the skin with a suspension of ultradeformable aggregates - and subsequent application of transcutaneous electric potential - can increase the final electrophoretic flux. We speculate that this might be due to the opening of channels in the skin by non-electrical means. Such an increase in 15 overall penetrability of the organ, which relies on more efficient, non-electrostatic channels opening, can be exploited subsequently to deliver loaded charged vesicles across the barrier. The latter are then pushed through pretreated skin by the transcutaneous electric potential applied under occlusion.

20 Last but not least, it is also plausible to postulate that, contrary to previous belief, large molecules can be delivered efficiently across the skin. Their delivery is made possible after macromolecular association with the charged ultradeformable carriers and involves transcutaneous electromotion of such unusually adaptable, protein-carrying transporters.

25 We further discuss some relevant properties of molecular aggregates/associates suitable for the use in conjunction with electrophoresis. We concentrate only on the complex bodies that can overcome transport barriers under the influence of a transbarrier electrical gradient of sufficient magnitude. We describe the basic experiments relevant for this phenomenon and interpret their results. We propose general conclusions useful 30 for the application of concepts advocated in this work in the widest possible sense. Particularly interesting, but not exclusive, is the use of our novel approach in the human

and veterinary medicine.

In the present invention, permeation denotes diffusive, concentration driven motion of molecules across a barrier. Penetration describes the non-diffusive motion of large
5 penetrants across a barrier; the process typically being associated with a penetration induced decrease in the barrier resistance (pore widening or channel opening).

A penetrant, consequently, is any entity comprising a single molecule or an arrangements of molecules too big to permeate through the barrier. A permeant, on the
10 other hand, is an entity that can permeate through the (semi-permeable) barrier. A penetrant in an external field experiences driving force proportional to the nominal penetrant size and to the applied field. Such a force may push the penetrant through the barrier, such as the skin, if the force is strong enough either to deform the penetrant or else to widen the passages in the barrier sufficiently to elude the problem of size
15 exclusion, or both. In the skin, for example, a transport-driving force must first intercalate the penetrant between cells to form channel wider than the effective penetrant size. (To achieve a high rate of penetrant transport, the effective penetrant size should be much smaller than the nominal penetrant size.) This goal is best achieved by the penetrants that are controllably and stress-dependently deformable. The average
20 diameter, the electrical charge and/or the adaptability in shape or size to the pores of the penetrant is selected so as to enable electromotion.

Electrical potential gradient (across a barrier) means an arbitrary potential difference of any sign or magnitude, unless otherwise specified. Specifically, it is not necessary to
25 place the potential generating electrodes directly on the barrier; any placement resulting in trans-barrier gradient is acceptable. "Potential difference" is used as a synonym for "potential gradient".

For further definitions, especially such pertaining to the highly deformable complex
30 bodies (aggregates) and their mechanism of action, as well as for the list of selected interesting agents, we explicitly refer to our issued or pending patents (DE 41 07 152,

PCT/EP91/01596, PCT/EP96/04526, DE 44 47 287). The same patents also contain detailed descriptions of the essential properties and characteristics of such aggregates.

In short, lipid aggregates should be able to compensate their deformation-induced, local stress (deformation energy) in order to be extremely deformable. This can be accomplished by adjusting their local composition to such a stress, which is only possible if aggregates comprise at least two components. The carrier ingredients are conveniently chosen so that the component which can sustain the deformation better is accumulated while the less adaptable component is diluted at the maximally stressed site. This results in a transient instability (metastability) which must be sufficiently short-lived not to compromise the aggregate integrity. Highly deformable vesicles named Transfersomes in the above mentioned patents (applications) were designed specifically to meet this need and to comply with the requirements for aggregate ultradeformability.

Preparation temperature is normally chosen in the 0 to 95 °C range. Preferably, one works in the temperature range 18-70 °C, most frequently at temperatures between 15 and 45 °C. On the skin, 32 °C are normally measured. Other temperature ranges are possible, however, most notably for the systems containing freezable or non-volatile components, cryo- or heat-stabilizers, etc.

If required to maintain the integrity and the desired properties of individual system components, carrier formulations can be stored in cold (e.g. at 4°C), with or without an associated active. Manufacturing and storage under an inert atmosphere, e.g. under nitrogen, is also possible and sometimes sensible. The shelf-life of (drug-loaded) carrier formulation, moreover, can be extended by using little unsaturated substances, by the addition of antioxidants, chellators, and other stabilizing agents, or by the ad hoc preparation from a freeze dried or dry mixture.

In the majority of cases the application is done at ambient temperature. An administration of useful suspension and potential application at lower or higher

temperatures are also possible. They make particular sense with the formulations comprising from synthetic substances which are rigid between the room and skin or other barrier temperature.

- 5 Formulations for the use in conjunction with electrophoresis can be processed at the site of application. For lipid vesicles, both charged and uncharged, examples are given in our previous german patent application and in the handbook on 'Liposomes' (Gregoriadis, G., Hrsg., CRC Press, Boca Raton, Fl., Vols 1-3, 1987), in the monography 'Liposomes as drug carriers' (Gregoriadis, G., Hrsg., John Wiley & Sons, 10 New York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R., Oxford-Press, 1989). If required, any suspension of drugs can also be diluted or concentrated (e.g. by ultracentrifugation or ultrafiltration) just before application; additives can also be given into a formulation at this time or before. After any system manipulation, the carrier characteristics should be checked and, if required, readjusted.

15

- This invention concerns a preparation comprising penetrants formed by single molecules or by arrangements of molecules, in which said penetrants are capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to 20 the pores. In this the penetrants are capable of transporting agents through the pores in the barrier. When the penetrants enter the pores a pore widening and channel opening by the penetrants results. Therefore, alternatively, agent permeation through the (now opened or widened) pores in the barrier is enabled subsequently to penetrant entry into said pores. The average diameter and the adaptability of said penetrants are selected, and 25 said penetrants and / or said agents are provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or in the alternative case agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability

30

According to the invention it is preferred if said penetrant is provided with sufficient

electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being
5 selected to achieve, and in case, control said transport through the barrier under the influence of the electrical driving force.

It further is preferred if said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores
10 in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force

15 Furthermore it is preferred if said penetrant is capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their permeation through the pores of the barrier subsequent to entry of said penetrant into said pores.

20 Said electrically charged penetrants or the charged association of penetrant and agent have preferably an average diameter which is greater (by at least the factor of 2) than the average diameter of the pores of the barrier.

25 In a particular embodiment of the invention a preparation is described which is characterized by the fact that the electrically charged penetrant is formed by an electrically charged single molecule or an arrangement of electrically charged molecules and is associated with one or several charged or uncharged agent molecules.

30 As a variation the above mentioned penetrant is formed by an electrically neutral single molecule or an arrangement of electrically neutral molecules and is associated with at

least one electrically charged agent, the quantity of electrical charges being sufficient to enable transport.

In a preferred embodiment of the invention said penetrants are suspended or dispersed
5 in a liquid medium and comprise arrangements of molecules in the form of minute fluid droplets surrounded by a membrane-like coating of one or several layers of at least two kinds or forms of amphiphilic substances with a tendency to aggregate, said at least two substances differing by at least a factor of 10 in solubility in the, preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble
10 substance or the average diameter of hetero-aggregates comprising both said substances is smaller than the average diameter of homo-aggregates of the less soluble substance.

It turns out to be advantageous if the more soluble substance is the agent to be transported through the barrier, and has a propensity to form common larger structures
15 with the less soluble substance. The common structure may comprise a physical or chemical complex of the substances.

According to the invention it is convenient if the more soluble substance tends to solubilize the penetrant droplet and the content of this substance is up to 99 mol% of the
20 concentration required to solubilize the droplet, or else corresponds to up to 99mol% of the saturating concentration in the unsolubilized droplet, whichever is higher.

It is preferred if the content of the more soluble substance is below 50 %, especially below 40 % and most preferably below 30 %, of the respective solubilizing
25 concentration of said substance.

According to the invention the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.

30

It is advantageous if the less soluble self-aggregating substance is a lipid-like substance

and the more soluble substance is a surfactant.

It is preferred if the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.

It is also preferred if the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.

According to the invention it is further advantageous if the average net surface charge density on a droplet is between 0.05 Cb m^{-2} (Coulomb per square meter) and 0.5 Cb m^{-2} , preferably between 0.075 Cb m^{-2} and 0.4 Cb m^{-2} , and particularly preferably between 0.10 Cb m^{-2} and 0.35 Cb m^{-2} .

It is preferred if the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

It is also preferred if the weight amount of droplets in formulations for the use on human or animal mucosa is 0,0001 to 30 weight-%.

Specific embodiments of the invention are disclosed in which the agent is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and/or bronchospasmolyticum, an antibioticum, antidrepressivum and/or antipsychoticum, an antidiabeticum, an antidot, antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an antihistaminicum, antihypertonicum, an antihypotonicum,

anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardiotonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antinflue agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance such as an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter (agonist) for the activity of any of the above-mentioned agents or any combination of such agents.

It is particularly advantageous if the liquid medium characteristics, especially the concentration and the composition of the supporting electrolyte, are selected so as to enable and / or control the rate or the efficiency of transport of the penetrant through the pores of the barrier.

According to the invention the supporting electrolyte, in particular a buffer, is selected among monovalent (1:1) or other low valency electrolytes, with the bulk concentration preferably below 150 mM, more preferably below 100 mM, even more preferably below 50 mM, and particularly preferably up to 10 mM.

Further, according to the invention a method for effecting the electrically driven transport of said penetrants and associated molecules through the pores in a barrier, as above defined, is provided which is characterized by the fact that a sufficient electrical potential is applied across the barrier.

According to the invention the electrodes used to generate the electrical potential across the barrier are located on opposite sides or on the same side of the barrier and are arranged so as to ensure that most of the resulting electrical current will flow across the barrier.

5

It is preferred if the applied electrical potential value is chosen to be below 30 V, more often below 15 V, and even more preferably below 10 V, per cm^2 of the barrier surface.

It is advantageous if the current driven across the barrier by the applied electrical potential is in the physiologically tolerable range, typically below 2 mA cm^{-2} ,

10 preferably below 1 mA cm^{-2} , more preferably below 0.6 mA cm^{-2} and most preferably up to 0.4 mA cm^{-2} .

Further it is preferred if the electrode size is less than 200 cm^2 , more preferably below 100 cm^2 , especially below 50 cm^2 , most preferably below 10 cm^2 , or even below 5 cm^2 .

15

According to the invention the electrically conductive material on or of the electrodes comprises at least one metal, in particular selected from precious metals, such as silver or palladium, and/or biocompatible salts or chemical complexes of such metals, preferably the biocompatible chlorides, and most preferably silver chloride.

20

It is advantageous if at least one the electrode compartment is loaded with electrically charged penetrants.

It has also been shown that it is advantageous if the electrode is loaded at the application
25 site or earlier.

It then is preferred if the electrode is loaded shortly before application, preferably within 360 min, more preferably within 60 min and even more preferably within 30 min.

30 In a preferred embodiment of the invention the electrode is loaded with the electrically charged penetrant pre-associated with molecules to be transported, in particular

(biologically active) agents.

In another preferred embodiment of the invention the electrode is loaded with the penetrant and the molecules to be transported, in particular agents, that associate
5 therewith during or after said loading.

In preferred embodiments of the invention one or more programmable, preferably small, hand-held or self-supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied
10 electric potential.

It is advantageous if different treatment areas are selected to control the transport.

In another preferred embodiment of the invention the barrier is pretreated before
15 initiating the electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

20

It is preferred if the charged or uncharged penetrants used to pre-treat the barrier are similar or identical with those employed for the subsequent electrically driven transport.

It is advantageous if the charged or uncharged penetrants are non-occlusively applied for
25 up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to the initiation of electrically driven transport of charged penetrants and/or permeants across the barrier. It shall be emphasized that it is a characteristic feature of the present invention that the electrically driven transport of permeants, i.e. any entity being capable
30 to permeate through the pores in the barrier, may be enhanced by a pre-treatment of the barrier as above described before initiating the electrically driven transport of the

permeant.

It further is advantageous if the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

Hereinafter, several illustrative examples of the invention's systems and methods are given; it will be understood that these neither define nor imply limits of this invention. All temperatures are in degree Celsius, carrier sizes are in nanometers, ratios and percentages are given in molar units, unless stated otherwise. Standard SI units are used otherwise in the text.

EXAMPLES

General experimental setup and sample preparation

Highly adaptable charged aggregates in the majority of cases studied in this work comprised anionic dioleoylphosphatidylglycerol (DOPG). Additional lipids with detergent or surfactant-like properties (typically the non-ionic Tween 80) were incorporated into lipid bilayers to increase the membrane flexibility. Increasing surfactant-to-lipid ratio made the vesicular aggregates more and more deformable, up to the concentration at which membrane stability was negatively affected by the detergent.

The total lipid concentration was typically 5 w-% and typically diluted to 0.5 %, unless stated otherwise. The bulk phase included buffering ingredients (10 mM) as well as, in some cases, dilute electrolyte (NaCl).

Laboratory made platinum electrodes were used in commercial glass-holders (Crown

Glass, Inc, USA), fixed to the device with a metal clamp. Hard-plastic covers provided with two openings for filling and sampling were pressed tight (sealed with O-rings) on the holder. During experiments, one of these openings was always open to let gas produced by water hydrolysis escape. This should, and has, prevented the bursting of the membrane/holder arrangement.

Freshly cleaned electrodes were separated from the receiving fluid with a microporous membrane (10 nm on the blank side and, for example, 30 nm on the test side). On the donor side, the filling volume was substantially bigger (1.2 mL) than on the blank side (14.5 mL), where the electrode was kept as close to the barrier as possible. The holder was used in a horizontal position to permit stirring of the receiver fluid. Stirring was achieved with a small magnetic bar that revolved on top of the tested barrier. The microporous barrier served as a surrogate or "artificial" skin, for the purposes of this study.

Electrical boundary conditions were defined and maintained with a constant current source (Phoresor: Iomed, Salt Lake City, USA; typical error: 0.1 mA) or a constant voltage source (Siemens, Munich, Germany; typical error: 1 mV). The test suspension was in contact with the cathode, whereas the blank sample volume was contacted by the anode.

The receiving fluid contained charged polymers (alginic acid: 0,25 w-%). These buffering polymers were first dissolved in salt-free water from an Elgastat purification unit (ELGA, UK) and then adjusted to the desired pH range (between 7 and 7.3) by titration with 0.01 N sodium hydroxide. To avoid changes in the mixed lipid vesicles composition, the fluid in the receiver compartment also contained 10^{-5} M of the most soluble vesicle component, that is, the critical micelle concentration of Tween 80. Benzyl alcohol (0.5 volume %) was added to prevent microbial system contamination during the experiments. The receiver fluid was forced by a peristaltic pump to circulate through the cuvette (placed in a fluorimeter) and to pass through the sampling cell into which a pH electrode was inserted. All experiments reported here were run at 37 degrees

Celsius.

Readings were taken continuously. The data were transformed into an electronically analysable and storable file using a XT-IBM micro-computer, equipped with an AD-converter and our own dedicated software.

For the data comparison we focused on the starting period, during which the electrical boundary conditions changed by a few percent only. The changes were estimated as good as possible and used to assign the errors shown in some figures.

10

To avoid false positive results, the completeness of label-aggregate association was confirmed early during the experimental work. To determine the relative amount of surfactant-solubilized label in the small mixed lipid micelles or in other kind of complexes, various suspensions were tested. This was done by pushing suspension through the membranes with 10 nm pores. Resulting fluxes were typically very small for the DPH labelled suspension. The corresponding flux values, nevertheless, were subtracted from the final flux of vesicles (that do not cross 10 nm pores). In experiments with epidermis, Rho-DHPE was used as the fluorescent label. Rho-DHPE is still highly lipophilic, but more soluble than DPH. Background signals with the former label were therefore higher than in the case of DPH-labelled vesicles and are shown in the figures directly rather than after subtraction from the other data.

20

Examples 1-2:

Aggregate charge effect

Uncharged highly deformable vesicles:

274 mg phosphatidylcholine (SPC)

226 mg Tween 80 (Tw80)

0.1 mol-% DPH (relative to SPC)

99.5 mL phosphate buffer, 10 mM, pH 7 - 7.3

Vesicle/pore size ratio: 3.3

Charged highly deformable vesicles:

274 mg phosphatidylglycerol (DOPG⁻, as above)

226 mg Tween 80

0.1 mol-% DPH (relative to SPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.7

Electrical current: 1.2 mA (current density: 0.279 mA/cm²)

Preparation of test suspension. The lipid mixture was suspended in dilute electrolyte. A sterile glass container containing crude lipid suspension was covered tightly and stirred magnetically for 3 days at room temperature. To narrow down the vesicle size distribution, the suspension was sequentially extruded through polycarbonate membranes of Nucleopore type with a nominal pore size of 400 nm, 100 nm and 50 nm, respectively. This was done at least 20 times. Vesicle suspension was then frozen and thawed 5 times at -70°C and + 50°C, respectively. To get the desired final vesicle size, suspension was re-extruded, 4 times through a 100 nm filter at 0.7 MPa. Finally, the suspension of highly deformable vesicles was sterilized by filtration through a sterile filter with 200 nm pores (Millipore) and stored at 4 °C.

Electrophoretic measurements. First, the background diffusion of label molecules
5 was determined. This was done for several hours without applying an electrostatic potential. Next, constant electric current was set and maintained across the barrier. During this second period, pH in various parts of the test system was monitored. In receiver compartment a digital pH meter was used whereas in donor compartment dipsticks were employed. Electrical potential difference across the barrier was permanently
10 assessed and recorded as well. Concurrently, the electrical barrier resistance was calculated (from the measured potential and current data using Ohm's law).

Fluorescence increase in the flow-through cuvette was monitored continuously.

Fluorescence increase was identified with the transported amount of material. This was done by using results from separate calibration measurements, during which known amounts of labelled suspensions were added directly into the receiving compartment.

- 5 The flow of lipophilic fluorescent label (DPH) across the barrier is believed to be representative of the electrically driven vesicles motion through the barrier. The transport data given in figure 1, consequently, correspond to cumulative effect of vesicle penetration through the barrier. The measured data reveal dramatic differences in the transport of charged and uncharged mixed lipid vesicles through "confining" pores
10 in the barrier. This clearly demonstrates the electromotive nature of aggregate transfer discovered and explored in this work.

- The lack of noncharged vesicle transport notwithstanding, the applied transbarrier potential does drive a flux of small charged molecules (chiefly ions) across the barrier,
15 as seen from the maintainance of constant current condition.

- Figure 2:** Time dependence of material and vesicle transport across a barrier with an applied electrical potential difference of 1.2 V, which gives rise to the trans-barrier electrical current of 0,279 mA cm⁻². Charged and uncharged,
20 zwitterionic, lipid vesicles were tested.

Examples 3-4:

Aggregate deformability effect

Conventional charged vesicles, liposomes:

500 mg phosphatidylglycerol (DOPG)

(prepared from from soy-bean phosphatidylcholine)

0.1 mol-% DPH (relative to DOPG)

99.5 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 2.9

Highly deformable charged vesicles:

274 mg phosphatidylglycerol (DOPG, as above)

226 mg Tween 80

0.1 mol-% DPH

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical current: 1.6 mA (current density: 0.381 mA cm^{-2})

Results obtained with conventional vesicles differ completely from the data measured with highly deformable vesicles: simple charged liposomes do not cross 30 nm pores in the barrier under the influence of an electrical (or, in fact, any other) driving force. The fact that no significant motion of the labelled molecules across the barrier is detected for at least 6 hours supports this conclusion. Conversely, the vesicles with a highly flexible and deformable, and thus better adaptable, membrane tend to move through the narrow pores in a barrier, when they are driven in the right direction by sufficiently strong transbarrier electrical potential difference.

10

Based on the fact that common lipid vesicles (liposomes) only cross the pores that are bigger than their own diameter, one would expect negligible aggregate penetration through the openings much smaller than the average vesicle diameter. Figure 1 contains unexpected and unprecedented data that put this expectation in question and require new concepts for explanation.

15

Results shown in figures 1 and 2 can be interpreted, for example, by generalizing the model of ultradeformable aggregate penetration described by the applicant (see e.g. Crit. Rev. Therapeutic Carrier Syst., 1997). The basic considerations for making such model modification are given in the introductory part of this application.

20

Figure 3: Vesicle transport (penetration) across a microporous barrier, deduced from the delivery of vesicle-associated DPH fluorescence, as function of time. Data suggests that liposomes that are ~3 times bigger than the pores cannot pass these obstacles, in contrast to the comparably large, but much more deformable, mixed lipid vesicles with composition that renders their membranes more flexible.

Examples 5-10:

Effects of vesicle size and electrical potential difference across the barrier

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

0.1 mol-% DPH (relative to DOPG)

226 mg Tween 80

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 5.2

Electrical driving force or parameters.

Current: 1.8 mA, 2.3 mA, 2.5 mA, 2.8 mA, 3 mA, 4 mA

Current: density: 0.429 mA/cm², 0.547 mA/cm², 0.595 mA/cm², 0.666 mA/cm²,

0.714 mA/cm², 0.952 mA/cm²

Experimental procedures were as described in examples 1-4. However, in this test series the effect of electrical potential difference was studied. This was first done by using relatively large vesicles which exceeded the average pore size by more than the factor of 5.

The results of this experimental series shown in figure 3 document the necessity of

applying at least 1 V potential difference across the barrier. 1.1 V to 1.2 V are sufficient to ensure significant transport of the highly deformable vesicles through 30 nm pores. To transport greater material amount through the barrier, trans-barrier potential in excess of 1.5 V is needed. Electrostatic potential differences of such magnitude then
5 results in rather high (opportunistic) electrical currents greater than 0.5 mA cm^{-2} .

It is therefore obvious that electro-passage of highly deformable vesicles through a barrier differs qualitatively from the simple electrophoresis or vesicle transport in the bulk. From Ohm's law one would predict that the electrically driven current will
10 increase linearly with the transport driving potential, commensurate to the system conductivity / inverse resistance. Such a linear dependence, and constant resistance, is indeed observed during the conventional electrophoresis. In this study, however, a strong nonlinearity was found. This cannot be a consequence of changing barrier properties. The data displayed in figure 3 thus suggest that vesicle capability to cross a
15 barrier increases with the applied potential. Similar report was made previously for the hydration-driven transport of highly deformable vesicles across a microporous barrier, and was explained with the mechanosensitivity of highly deformable mixed lipid membranes.

20 **Figure 4:** Temporal characteristics (upper panel) and potential sensitivity (lower panel) of the vesicles with an aggregate/pore size ratio of approximately 5.2, penetrating the transport barrier under influence of an external, transport driving electrical potential.

25

Examples 11-16:

Suspension characteristics:

As in examples 5-10, except for a decrease in

Vesicle/pore size ratio: 4.6

Electrical driving force or parameters.

Current: 1.4 mA, 1.6 mA, 1.8 mA, 2 mA, 2.5 mA, 3 mA

Current: density: 0.333 mA/cm², 0.381 mA/cm², 0.429 mA/cm², 0.476 mA/cm², 0.595 mA/cm², 0.714 mA/cm²

Experiments were done and analyzed as discussed in examples 1-11. Notable difference was the decreased relative vesicle size, however, which shifted minimum potential difference required for a substantial vesicle penetration across the barrier to 1.2 V (see figure 4). Even with the highest potential difference studied to date (1.7 V), no clear proof of the saturation of potential-dependent transport increase was obtained. Significant, albeit smaller fluxes were measured with the electrostatic potential differences above 0.8 V, however.

Figure 5: Characteristic time course (upper panel) and potential sensitivity (lower panel) of ultradeformable vesicle penetrating through the pores nearly 4.6 narrower than the average aggregate diameter.

Examples 17-28:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

99 mL phosphate buffer, 10 mM, pH 7

Vesicle/pore size ratio: 3.5

Electrical driving force or parameters.

Current: 0.4 mA, 0.6 mA, 0.8 mA, 1.2 mA,

1.4 mA, 1.5 mA, 1.6 mA, 2.3 mA, 3 mA,

3.5 mA, 4 mA

Current: density: 0.095 mA/cm², 0.143 mA/cm², 0.190 mA/cm², 0.286 mA/cm², 0.333 mA/cm², 0.357 mA/cm², 0.381 mA/cm², 0.547 mA/cm², 0.714 mA/cm², 0.833 mA/cm², 0.952 mA/cm²

Results. Proper pore penetration is observed when the transbarrier electrostatic potential difference is at least 1 V. Significant, albeit smaller fluxes are measured when the difference exceeds 0.8 V.

5

Transbarrier potential difference bigger than approximately 1.3 V appears to make the flux of DPH (and by inference, the transport of vesicles) less sensitive to changes in the electrical transbarrier driving potential. It is not entirely clear whether or not the diminished increase in penetration capability, measured with the highest explored potential difference, is diagnostic of saturation of the potential dependent changes in the vesicle transport (see examples 29-35), or else is simply due to the experimental irreproducibility. Data given in the middle panel of figure 5 circumstantially support the former interpretation: if the transport is not analyzed as a function of time but rather as a function of time required to bring certain number of non-confined ions across the barrier, all the curves measured with driving potentials higher than 1 V group closer together.

Figure 6: Effect of electrostatic potential difference on the transport of highly deformable, intermediate size vesicles passing 30 nm pores. Upper panel: time course of flux measured under the constant current conditions; middle panel: data as above, but with the time-axis normalized with relatively to the given electrical current; lower panel: capability of ultradeformable vesicles to penetrate pores of fixed-size by electromotion.

25 **Examples 29-35:**

Suspension characteristics:

As in examples 17-28. except for
Vesicle/pore size ratio: 2.6

Electrical driving force or parameters.

Current: 0.25 mA, 0.4 mA, 1 mA, 1.2 mA, 1.4 mA,
1.8 mA, 2.3 mA

Current: density: 0.060 mA/cm², 0.095 mA/cm²,
0.238 mA/cm², 0.286 mA/cm²,
0.333 mA/cm², 0.429 mA/cm², 0.548 mA/cm²

Test conditions in this experimental series were such that the exclusion criterium for the lipid vesicles motion across a barrier with the vesicle/pore size ratio of 2.6 was very weak. (It is known from previously published work by us (Cevc et al., Biochim. Biophys. Acta 1368, 201-215, 1998) and the others that size exclusion begins to govern the transport across microporous barriers when the penetrant/pore size ratio exceeds the value of 2. The flux of vesicle-associated label, consequently, was biphasic in this test series (cf. figure 6). Normalization of the time axis (see the middle panel of figures 5 and 6) does not group the curves together. Rather than this it makes the spread more uniform.

Initial slope of material transport curve measured for different transbarrier potential and current values is fairly constant. This is illustrated in lowest panel, which gives normalized slopes of the curves shown in upper panel. The "early part" of measured curves reveals little, if any, voltage dependence. In contrast, the later time flux characteristics (after approx. 1 hour) are indicative of a change in the system properties, which is seen when transbarrier voltage or current is sufficiently high (0.7 V and 0.225 mA cm⁻², respectively). The observed lag-time is fairly insensitive to the electrical current, as can be seen from the upper panel of figure 2, but does get somewhat shorter with increase in current/potential value.

Barrier penetrability can not increase significantly upon changing the applied voltage. It

is therefore more than probable that the above mentioned late penetrability change results from increased capability of lipid aggregates to pass the barrier. We interpret this difference as a sign of a moderately increased vesicle adaptability to pore narrowness.

The earlier transbarrier transport, on the other hand, is likely to be due to simple
5 electrophoresis of relatively tiny vesicles. Obviously, many such vesicles are small enough to cross the pores in a barrier, probably in the process of an electrically mediated (or supported) "diffusion".

Penetration capability data illustrated in the lower panel of figure 6 are diagnostic of
10 complete vesicle adaptability (maximum membrane flexibility), as can be seen from the fact that several high potential values are nearly the same.

Figure 7: Elektromotion of relatively small, highly deformable vesicles through 30 nm pores in a barrier. Upper panel: absolute penetration of vesicles, as
15 calculated from the measured DPH flux; middle panel: the same data as above, as a function of normalized time; lower panel: relative penetration capability of tested system (=DPH-derived vesicle flux per unit potential). Two different transport rates (ϕ_1 and ϕ_2) are seen, indicative of two different unerlaying transport phenomena.

20

Examples 36-40:

Effects of electrolyte concentration

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

Buffer as in previous examples

NaCl concentration (final): 1 mM, 10 mM, 20 mM, 50 mM, 100 mM

Vesicle/pore size ratio: 3.3

Electrical current: 1.2 mA; current density 0.286 mA cm^{-2}

In this test series we have shown that increasing supporting electrolyte concentration strongly affects the efficiency of electrically driven transport across a microporous barrier. High electrolyte concentrations typically support the salt transport but lower the transbarrier flux of aggregates. Above certain threshold concentration, which is believed
5 to depend on the barrier as well as penetrant properties, the added salt may bring the transport of large aggregates to a halt.

Comparison of material flux, ion current and driving electrical potential data measured in this and previous set of experiments provides a clue to explaining the salt-dependent
10 suppression of aggregate electromotion through the narrow pores. The relative contribution of small anions (here Cl^-) flow across the barrier is proportional to the bulk salt concentration. A lower driving potential, consequently, suffices to maintain a constant current across the barrier at higher salt concentrations (see lower panels in figure 7). Lower driving potential simultaneously lessens the ease of, and thus the
15 probability for, large penetrant adaptability and penetration capability (see upper right panel). The latter is the proviso for an efficient flow of aggregates, however, through the narrow pores. Below certain adaptability value, which is affected by the penetrant/pore size ratio, the deformability of aggregates therefore becomes so low that only insignificant transport takes place.

20

Addition of salt to the suspension of complex aggregates capable of very strong, stress driven deformation therefore detrimentally affects the transbarrier transport.

Figure 8: Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores in the presence of different salt solutions.
25 Upper left: transbarrier flow of DPH labelled vesicles; upper right: penetration capability of complex aggregates; lower left: electrical potential

that drives the constant current across a barrier as a function of time; lower right: transport driving electrostatic potential as function of bulk NaCl concentration.

5

Examples 41-50:Changes in the barrier properties and in the test system characteristics

Suspension characteristics:

As in examples 17-28

Electrical driving force or parameters.

As in examples 17-28

Figure 8 shows that the electrical resistance to electrophoretic motion across a barrier
10 increases nearly linearly with the applied electrical potential, but only in certain range.
The onset of material flux, in parallel, gets faster (see figure 5 for comparison). This
means that the lag-time becomes shorter with increasing transbarrier potential
difference. Below the "linear" range, which commences at approximately 1 V for the
tested suspension, only insignificant vesicle transport is observed. The tiny flow of
15 aggregate material is then hardly affected by the applied potential or by the changing
electrical current.

During experiments done in this test series, pH in the receiver compartment dropped by
approximately 1.5 to 1.8 units, nearly independent of the applied voltage. Over the first
20 hour of electrophoresis the change was smaller than 1 unit. (Such a variation was
considered to have had only a small, if any, effect on the vesicle electromotion.) In
parallel, the donor compartment pH became more alkaline by the corresponding
amount. This latter variation did not change the charge on the mixed lipid vesicles,
owing to the low $pK = 2.9$ of ionic PG in the membranes. Lipid degradation, which is
25 faster when the charged membranes diverge from their optimum at $pH \sim 7.1$, is believed

to have been insignificant during the first part of experiment at least.

Figure 9: Electrically driven transport of charged, highly deformable vesicles across an artificial barrier with 30 nm pores. Upper left: barrier electrical resistance; upper right: electrical potential required to drive constant electrical current across the barrier; lower left: pH value in the receiver compartment containing alginic acid; lower right: pH of suspension of highly flexible vesicles present in the donor compartment.

Data interpretation. When the rate of vesicle transport across a barrier is substantial, the electrical potential difference that needs to be applied to drive a constant electrical current through the pores first rapidly decreases and finally increases with time. We believe that the former phenomenon results from the redistribution of highly mobile ions in the system, especially in front of the electrodes and near the barrier. The secondary, and much slower increase, in our opinion, is largely due to the gradual pore clogging by the large or poorly deformable vesicles accumulated in front of the barrier.

Changes on or near the electrodes could partly explain the secondary changes in barrier penetrability / driving potential values. Especially with the high currents we often saw material (alginic acid?) precipitation near the reference electrode. Electrode surface also always turned brown during the course of an experiment; the higher was the applied potential or the bigger was the resulting current the more this was the case. Last but not least, hydrogen and oxygen evolution in the solution, which occasionally led to slight suspension foaming, also could have contributed to the above mentioned barrier resistance changes.

Examples 51-53:

Transport characteristics of the skin (epidermis)

Electrical parameters: as shown in figure 9

(Currents are given in the panels)

Electromotion through the epidermis in vitro can be used to study some of the characteristics of electrophoresis in vivo. The proviso for this is the use of sufficiently large and intact skin segments with a functional barrier. In order to obtain at least semi-quantitatively reliable data, such skin pieces must also be as thin as possible. Ideally, one would like to work with a mere barrier, that is, with the stratum corneum only. In practice it is impossible to achieve this task, owing to the fragility of the horny skin layer. The best that one can do then is to prepare thin but sufficiently extended pieces of the epidermis.

Electrical resistance of the epidermis is a good marker for the skin intactness. It is also diagnostic of any major changes in the barrier properties of the organ.

An example for the variable electrical resistance of the skin as a function of time during transepidermal electrophoresis is given in figure 9.

Figure 10:Electrical resistance of epidermis during serial electrophoresis experiments done in vitro.

20

The specific resistance of excised skin (originally somewhat higher than $10 \text{ k}\Omega \text{ cm}^{-2}$) always decreases with the cumulative current that has flown through the skin to approximately 10-20 % of starting value. This observation as well as the starting specific resistance value is comparable to the published information, which gives specific resistance values as below $20 \text{ k}\Omega \text{ cm}^{-2}$ for human and murine skin. The somewhat higher resistance decrease to approximately 10% of starting value could be due to the difference in total, cumulative current.

We observed no significant difference in electrical resistance, or its time and current variation, between the tested human and porcine skin samples.

30

Examples 54-57:**Barrier (epidermis) thickness effect:****Suspension characteristics:**

Total lipid (TL) content 0.5 w-% comprising:

274 mg phosphatidylglycerol (DOPG)

226 mg Tween 80

0.1 mol-% DPH (relative to DOPG)

Buffer as in previous examples

- Part A: Vesicle/pore size ratio: 3.3;
electrical current: 0 mA, 1.2 mA (current density 0.286 mA cm^{-2})
electrical potential: difference: 0 V, 2.0 V
- Part B: Vesicle/pore size ratio: 2.8
electrical current: 1.2 mA (current density 0.286 mA cm^{-2})
electrical potential: difference: 3.7 V, 5.4 V, and 7 V

- 5 **Results.** In first experiment (part A), an electrical current of approximately 0.3 mA cm^{-2} was shown to co-transport only a small amount of fluorescently labelled ultradeformable vesicles through the thick epidermis, prepared by heat-separation and 2 hours of trypsin action; only approximately 6 micrograms of material have passed through each square centimeter in approx. 4 hours. Then, a collapse in the skin barrier
- 10 resulted in strong decrease of electrical resistance of the skin and in a concomitant increase of material flow through the (probable) perforated organ.

- Further experiments (part B) were done with two skin preparation methods. 2 hours and 7 hours of enzymatic action were used for this purpose, which gave rise to rather thick
- 15 (5.4 V; 3.7 V) or thin (7 V) specimen, respectively. Moreover, slightly smaller vesicles were used than in part A. This latter difference notwithstanding, the results from repeat experiments have confirmed the trend observed in part A experiment. They also revealed the importance of skin thickness on the effective vesicle flux across the barrier.

After a lag-time of approximately 22 min the transport of ultradeformable vesicles across thin skin, as assessed by means of fluorescent label flux determination in part B, was substantial ($0.4 \text{ microgramms cm}^{-2} \text{ min}^{-1}$ or approx. $25 \text{ microgramms cm}^{-2} \text{ h}^{-1}$, see figure 11). Conversely, an order of magnitude smaller flux was measured with the two thicker epidermis samples. However, even in the case of high flux, saturation was observed. This could result from the clogging of pores in the skin, which are available in limited number, especially under conditions as used in this test.

Figure 11: A) Electrically driven transport of ultradeformable vesicles across human epidermis (upper panel), electrical resistance of the barrier (middle panel) and pH in receiver compartment (lower panel) during an electrophoretic experiment.
B) Transport of charged vesicles through thin or thick epidermal samples with an applied electrical potential.
Inset gives the corresponding rate of penetration.

Example 58:

Suspension characteristics:

Total lipid (TL) content 0.5 w-% comprising:
274 mg phosphatidylglycerol (DOPG)
226 mg Tween 80
 ^3H -DPPC (relative to DOPG)
Buffer as in previous examples
Vesicle/pore size ratio: 3.5

Electrical characteristics:

Electrical current: 0 mA, 0.2 mA
(current density: 0, 0.048 mA cm^{-2})
Electrical potential: difference: 0.5 V

The barrier for this test was prepared by acting with trypsin for 7 hours on a heat-

separated murine epidermis sample. Instead of using fluorescent labels, radioactive phospholipids were used. Consequently, the samples were taken from receiver fluid manually and the readings were made with a beta-counter. Further difference between this and previous experiments was the use of murine, rather than human, epidermis and
5 the relatively low electric current.

After a lag-time of approximately 30 min, constant vesicle transport was seen to commence. The rate of lipid transfer across the barrier, calculated from the following 90 min linear period, was approximately 4 microgramms per hour and cm^{-2} . Thereafter, no
10 significant further transport was observed, in accordance with the findings from the other measurements with the non-radioactive labels.

This experiment clearly documents that charged carriers (here due to the presence of DOPG) can be used to electro-transport uncharged substances (here ^3H -DPPC) across a
15 barrier with an applied electrical potential difference.

Figure 12: Electro-transport of uncharged molecules associated with charged, ultradeformable vesicles across murine epidermis in vitro.

CLAIMS

1. A preparation comprising penetrants formed by single molecules or by arrangements of molecules, said penetrants being capable of penetrating the pores of a barrier even when the average diameter of said barrier pores is less than the average diameter of said penetrants, since the penetrants are adaptable to the pores, and said penetrants being capable of transporting agents through said pores, or enabling agent permeation through said pores after the penetrants have entered said pores; the average diameter and the adaptability of said penetrants being selected, and said penetrants and / or said agents being provided with sufficient electrical charges, to enable and / or control agent transport through said pores by said penetrants, or agent permeation through said pores after penetrant entry into said pores, under the influence of a suitable electrical driving force, said selection at the same time maintaining sufficient penetrant stability.

15

2. Preparation according to claim 1, characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could, in the absence of an electrical driving force, not readily penetrate the barrier pores; the average diameter, the kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent, being selected to achieve, and in case, control said transport through the barrier under the influence of the electrical driving force.

20

25 3. Preparation according to claim 1, characterized in that said penetrant is provided with sufficient electrical charges, at least when associated with an agent, and the penetrant could penetrate the barrier pores in the absence of an electrical driving force; the average diameter, kind and amount of electrical charges and / or the adaptability of the electrically charged penetrants or the charged associations of penetrant and agent being selected to provide control of the agent transport through the barrier under the influence of an electrical driving force.

30

4. Preparation according to any one of claims 1 to 3, said penetrant being capable of penetrating said pores under the influence of a suitable driving force, which may be an electrical driving force when the penetrant is suitably electrically charged, and the agents being sufficient electrically charged to enable and / or control their
5 permeation through the pores of the barrier subsequent to entry of said penetrant into said pores by means of an electrical driving force.

5. Preparation according to any one of claims 1 through 4,
characterized in that the average diameter of the electrically charged penetrants or the
10 charged association of penetrant and agent, is greater (by at least the factor of 2) than the average diameter of the pores of the barrier.

6. Preparation according to any one of claims 1 through 5,
characterized in that the penetrant is formed by an electrically charged single molecule
15 or an arrangement of electrically charged molecules and is associated with one or several charged or uncharged agent molecules.

7. Preparation according to any one of claims 1 through 5,
characterized in that the penetrant is formed by an electrically neutral single molecule
20 or an arrangement of electrically neutral molecules and is associated with at least one electrically charged agent, the quantity of electrical charges being sufficient to enable transport.

25

30

8. Preparation according to claim 6 or 7,
characterized in that the penetrants are suspended or dispersed in a liquid medium and
comprise arrangements of molecules in the form of minute fluid droplets surrounded by
a membrane-like coating of one or several layers of at least two kinds or forms of
5 amphiphilic substances with a tendency to aggregate, said at least two substances
differing by at least a factor of 10 in solubility in the, preferably aqueous, liquid
medium, such that the average diameter of homo-aggregates of the more soluble
substance or the average diameter of hetero-aggregates comprising both said substances
is smaller than the average diameter of homo-aggregates of the less soluble substance.

10

9. Preparation according to claim 8,
characterized in that the more soluble substance is the agent to be transported through
the barrier, and has a propensity to form common larger structures with the less soluble
substance.

15

10. Preparation according to claim 9,
characterized in that the common structure comprises a physical or chemical complex
of the substances.

20 11. Preparation according to claim 8, 9 or 10,
characterized in that the more soluble substance tends to solubilize the penetrant
droplet and the content of this substance is up to 99 mol% of the concentration required
to solubilize the droplet, or else corresponds to up to 99 mol% of the saturating
concentration in the unsolubilized droplet, whichever is higher.

25

12. Preparation according to claim 11,
characterized in that the content of the more soluble substance is below 50 %,
especially below 40 % and most preferably below 30 %, of the respective solubilizing
concentration of said substance.

30

13. Preparation according to claim 11,
characterized in that the content of the more soluble substance is below 99 %, preferably below 80 % and most preferably below 60 % of the saturation concentration of said substance in the droplet.

5

14. Preparation according to any one of claims 8 through 13,
characterized in that the less soluble self-aggregating substance is a lipid-like substance and the more soluble substance is a surfactant.

10

15. Preparation according to any one of claims 8 through 14,
characterized in that the average diameter of the penetrant is between 40 nm and 500 nm, preferably between 50 nm and 250 nm, even more preferably between 55 nm and 150 nm and particularly preferably between 60 nm and 120 nm.

15

16. Preparation according to any one of claims 8 through 14,
characterized in that the average diameter of the penetrant is 2 to 25 times bigger than the average diameter of the pores in the barrier, preferably between 2.25 and 15 times bigger, even more preferably between 2.5 and 8 times bigger and most preferably between 3 and 6 times bigger than said average pore diameter.

20

17. Preparation according to any one of claims 8 through 16,
characterized in that the average net surface charge density on a droplet is between 0.05 Cb m⁻² (Coulomb per square meter) and 0.5 Cb m⁻², preferably between 0.075 Cb m⁻² and 0.4 Cb m⁻², and particularly preferably between 0.10 Cb m⁻² and
25 0.35 Cb m⁻².

30

18. Preparation according to any one of claims 8 through 17,
characterized in that the weight amount of droplets in formulations for use on human or animal skin is 0.01 to 40 weight-% of the total preparation mass, in particular
30 between 0.1 and 30 weight-%, and particularly preferably between 5 and 20 weight-%.

19. Preparation according to any one of claims 8 through 17,
characterized in that the weight amount of droplets in formulations for the use on
human or animal mucosa is 0,0001 to 30 weight-%.

5 20. Preparation according to any one of claims 8 through 19,
characterized in that, the agent is an adrenocorticostaticum, an adrenolyticum, an
androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or
analgesicum, an analepticum, an antiallergicum, antiarrhythmicum,
10 antiarteroscleroticum, antiasthmaticum and / or bronchospasmolyticum, an
antibioticum, antidrepressivum and / or antipsychoticum, an antidiabeticum, an antidot,
antiemetikum, antiepilepticum, antifibrinolyticum, anticonvulsivum or
anticholinergicum, an enzyme, coenzyme or a corresponding enzyme inhibitor, an
antihistaminicum, antihypertonicum, an antihypotonicum, anticoagulant,
antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Parkinson, an
15 antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory
analepticum or a respiratory stimulant, a broncholyticum, cardi tonicum,
chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-
blocker, a glucocorticoid, an antinflue agent, a haemostaticum, a hypnoticum, an
immunoglobuline or its fragment or any other immunologically active substance such as
20 an immunomodulator, a cytokine, etc., a bioactive carbohydrate(derivative), a
contraceptive, an anti-migraine agent, a corticosteroid, a muscle relaxant, a narcoticum,
a neurotherapeutic agent, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a
(poly)peptide(derivative), an opiate, an ophthalmicum, a (para)-sympaticomimeticum or
(para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a
25 mydriaticum, a psychostimulant, a rhinologicum, a sleep-inducing agent, a sedating
agent, a spasmolyticum, tuberlostaticum, urologicum, a vasoconstrictor or vasodilatator,
a virustaticum, a wound-healing substance, an inhibitor (antagonist) or promoter
(agonist) for the activity of any of the above-mentioned agents or any combination of
such agents.

30

21. Preparation according to any one of claims 8 through 20,
characterized in that the liquid medium characteristics, especially the concentration
and the composition of the supporting electrolyte, are selected so as to enable and / or
control the rate or the efficiency of transport of the penetrant through the pores of the
5 barrier.

22. Preparation according to any one of claims 8 through 21,
characterized in that the supporting electrolyte, in particular a buffer, is selected
among monovalent (1 : 1) or other low valency electrolytes, with the bulk concentration
10 preferably below 150 mM, more preferably below 100 mM, even more preferably below
50 mM, and particularly preferably up to 10 mM.

23. A method for effecting the electrically driven transport of penetrants and
associated molecules through the pores in a barrier, as defined in any one of the
15 preceding claims,
characterized in that a sufficient electrical potential is applied across the barrier.

24. Method according to claim 23,
characterized in that the electrodes used to generate the electrical potential across the
20 barrier are located on opposite sides or on the same side of the barrier and are arranged
so as to ensure that most of the resulting electrical current will flow across the barrier.

25. Method according to claims 23 or 24,
characterized in that the applied electrical potential value is chosen to be below 30 V,
25 more often below 15 V, and even more preferably below 10 V, per cm² of the barrier
surface.

26. Method according to claims 23 or 24,
characterized in that the current driven across the barrier by the applied electrical potential is in the physiologically tolerable range, typically below 2 mA cm⁻², preferably below 1 mA cm⁻², more preferably below 0.6 mA cm⁻² and most preferably
5 up to 0.4 mA cm⁻².

27. Method according to any one of claims 23 through 26,
characterized in that the electrode size is less than 200 cm², more preferably below 100 cm², especially below 50 cm², most preferably below 10 cm², or even below
10 5 cm².

28. Method according to any one of claims 23 to 27,
characterized in that the electrically conductive material on or of the electrodes comprises at least one metal, in particular selected from precious metals, such as silver
15 or palladium, and / or biocompatible salts or chemical complexes of such metals, preferably the biocompatible chlorides, and most preferably silver chloride.

29. Method according to any one of claims 23 to 28,
characterized in that at least one electrode compartment is loaded with electrically
20 charged penetrants.

30. Method according to claim 29,
characterized in that the electrode is loaded at the application site or earlier.

25 31. Method according to claims 29 or 30,
characterized in that the electrode is loaded shortly before application, preferably within 360 minutes, more preferably within 60 minutes and even more preferably within 30 minutes.

30 32. Method according to claims 29, 30 or 31,
characterized in that the electrode is loaded with the electrically charged penetrant

pre-associated with molecules to be transported, in particular (biologically active) agents.

33. Method according to claims 29, 30 or 31,
5 **characterized in that** the electrode is loaded with the penetrant and the molecules to be transported, in particular agents that associate therewith during or after said loading.

34. Method according to any one of claims 23 through 33,
characterized in that one or more programmable, preferably small, hand-held or self-
10 supported, for example wrist-watch like, devices for single or repeated use are employed to control the polarity, magnitude and / or time-dependence of applied electric potential.

35. Method according to any one of claims 23 through 34,
characterized in that different treatment areas are selected to control the transport.

15 36. Method according to any one of claims 23 through 35,
characterized in that the barrier is pre-treated, before initiating the electrically driven transport of charged penetrants, by a non-occlusive application of suitable penetrants on the modifiable barrier, especially formed by human or animal skin, to increase the
20 number or width of penetratable pores in the barrier subsequently to be used for the electrically driven transport across said pre-treated skin barrier.

37. Method according to claim 36,
characterized in that the charged or uncharged penetrants used to pre-treat the barrier
25 are similar or identical with those employed for the subsequent electrically driven transport.

38. Method according to claims 36 or 37,
characterized in that the charged or uncharged penetrants are non-occlusively applied
30 for up to 24 hours or even longer, typically for up to 12 hours, especially up to 3 hours, or more preferably for less than 1.5 hours, and in case even for less than 30 min, prior to

the initiation of electrically driven transport of charged penetrants and / or permeants across the barrier.

39. Method according to any one of claims 23 to 38,
- 5 **characterized in that** the transportation rate, i.e. the flux, of charged penetrants through the barrier pores is determined as a function of the applied electrical potential or of the electrical current across the barrier, and the function thus found is then employed to optimize the preparation or application.

10

15

20

25

30

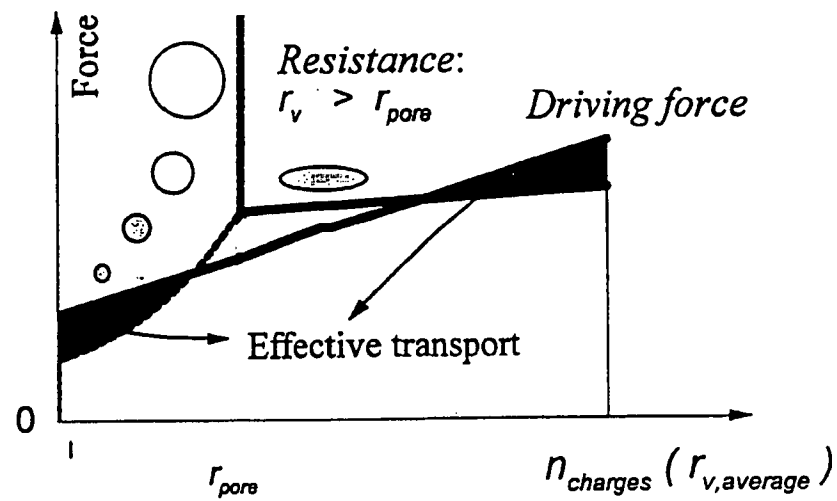
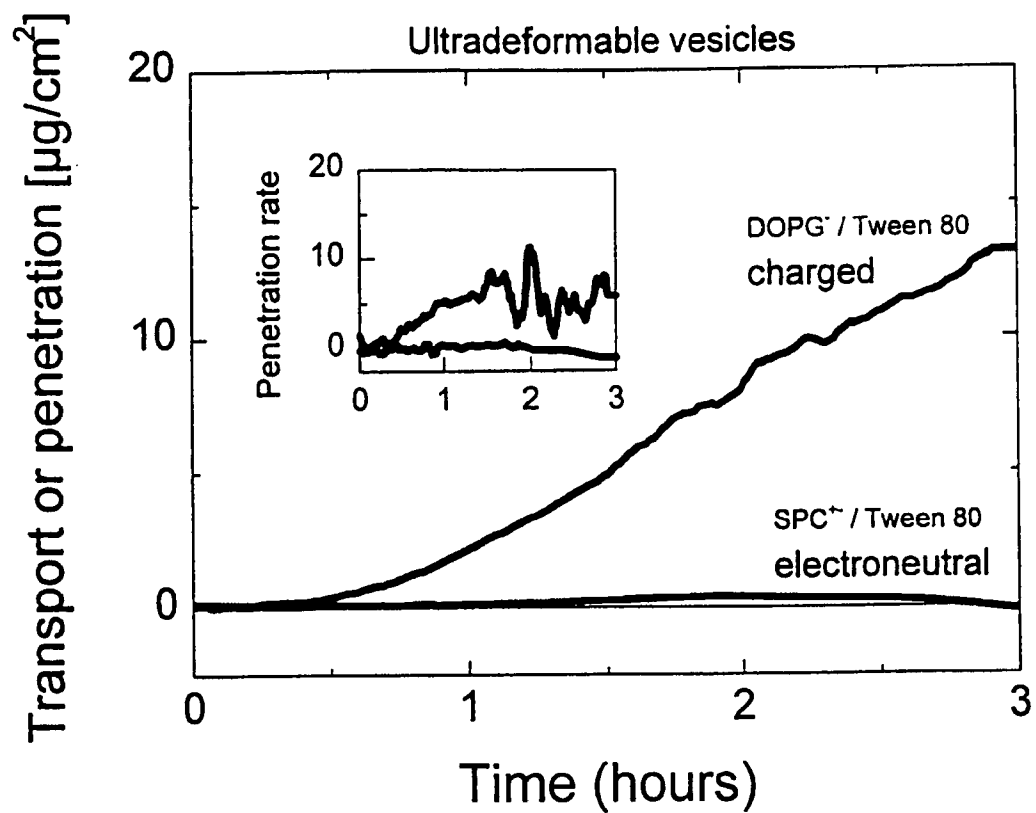
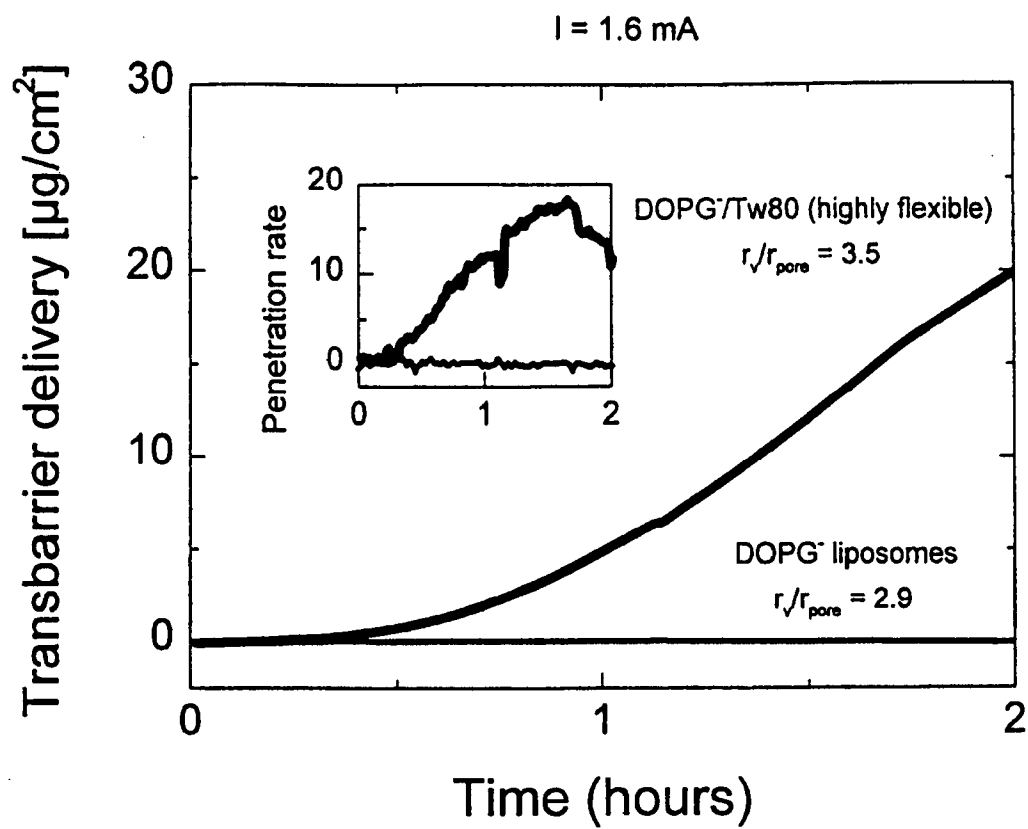


Figure 1





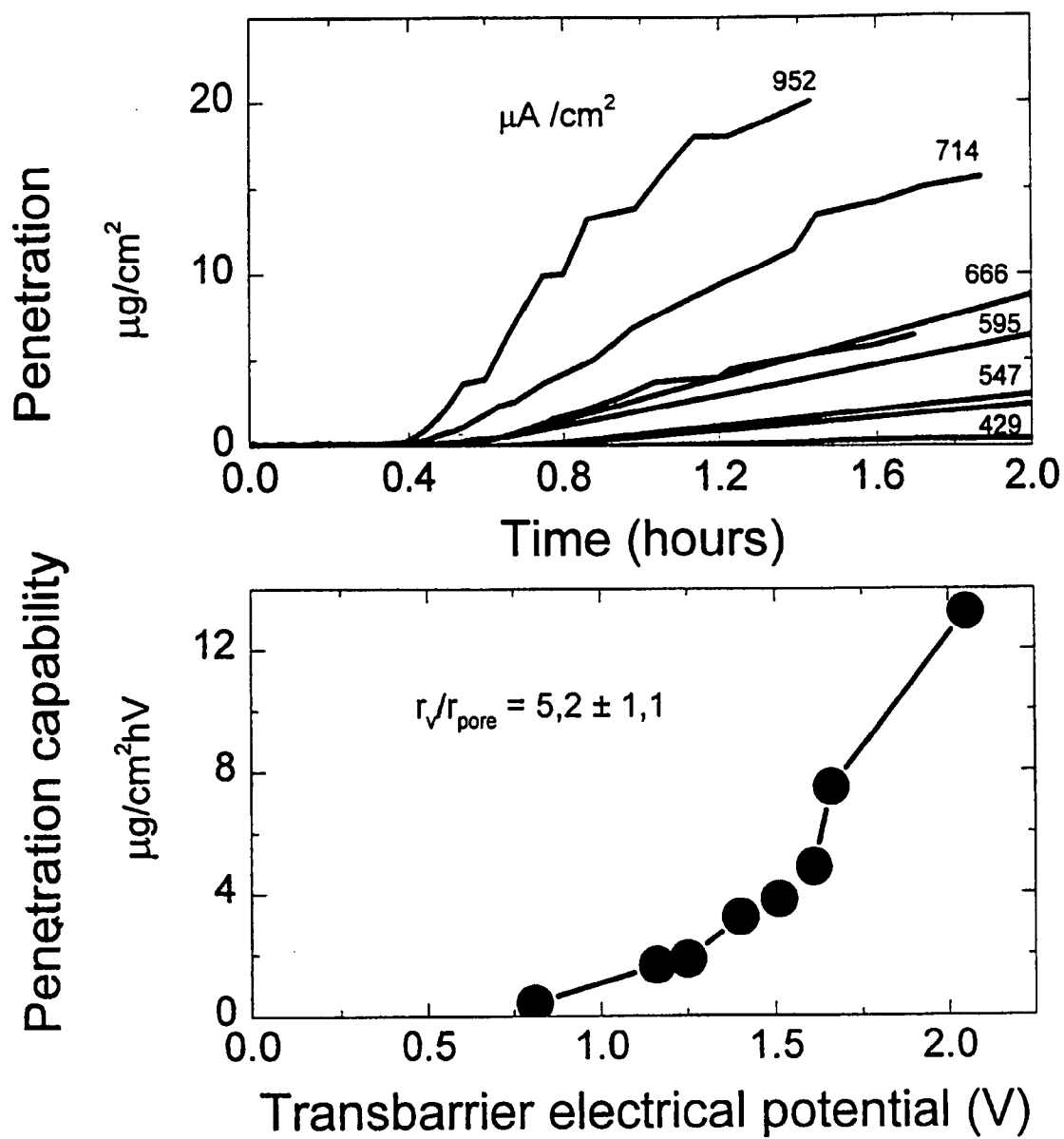


Figure 4

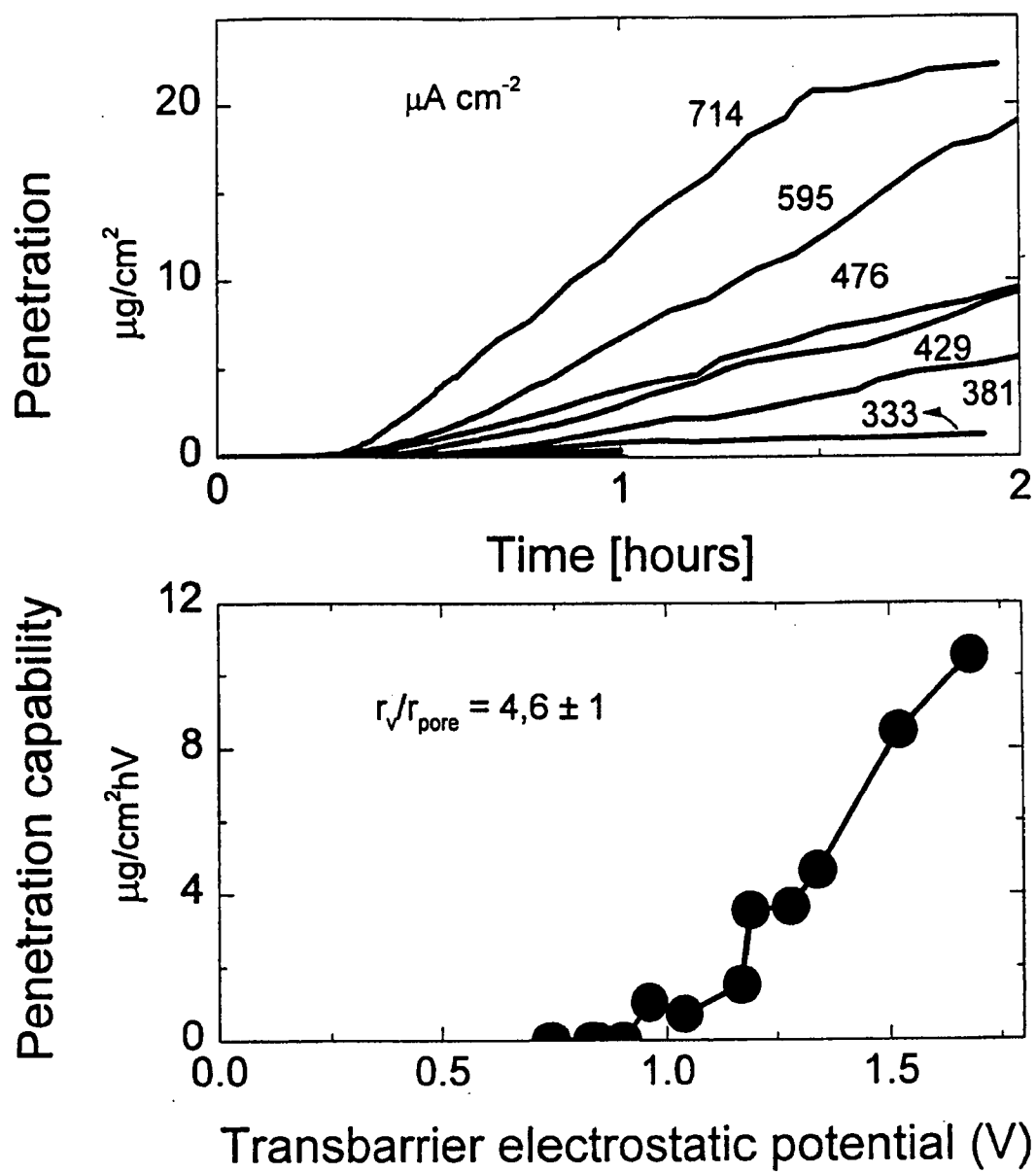


Figure 5

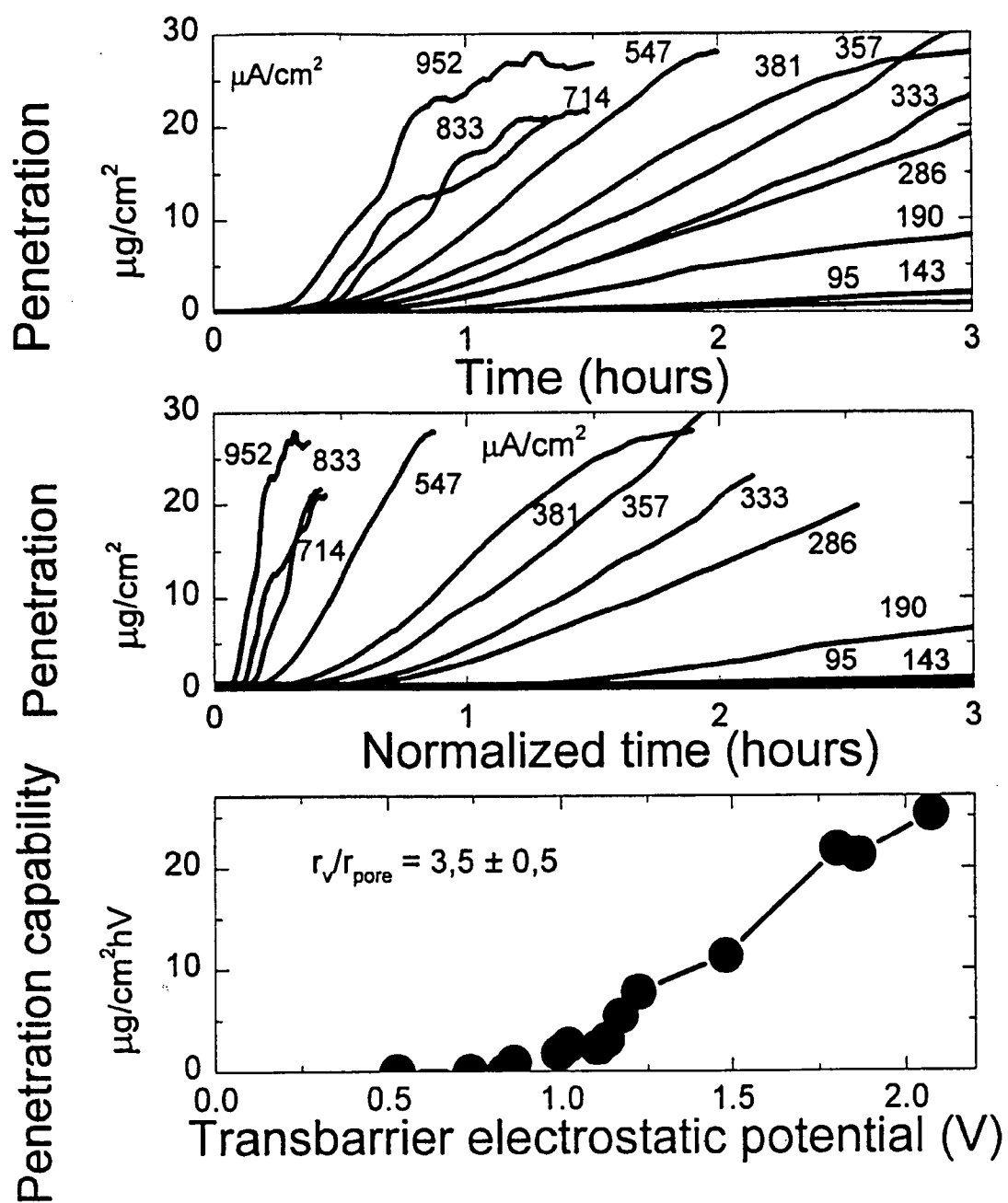


Figure 6

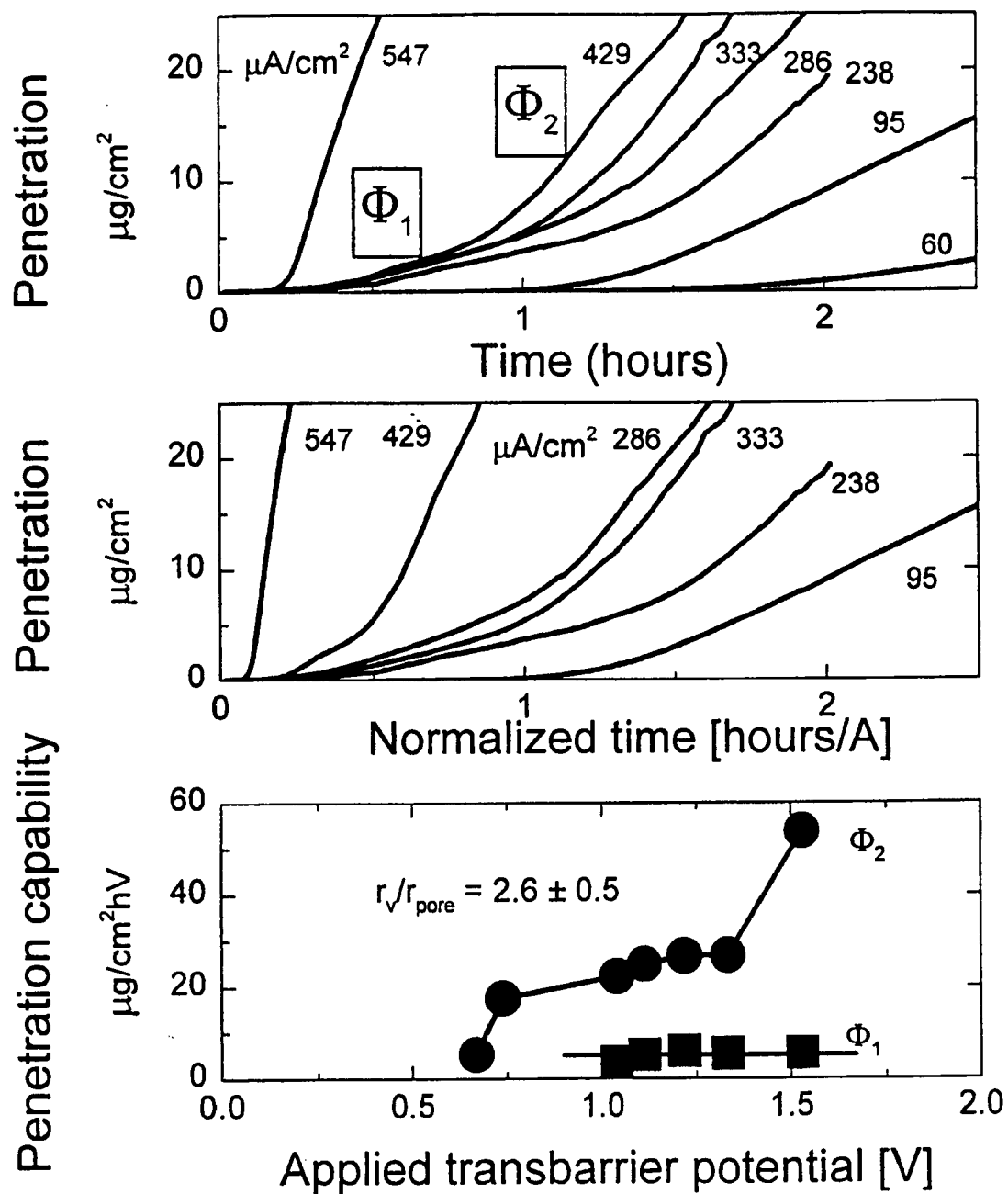


Figure 7

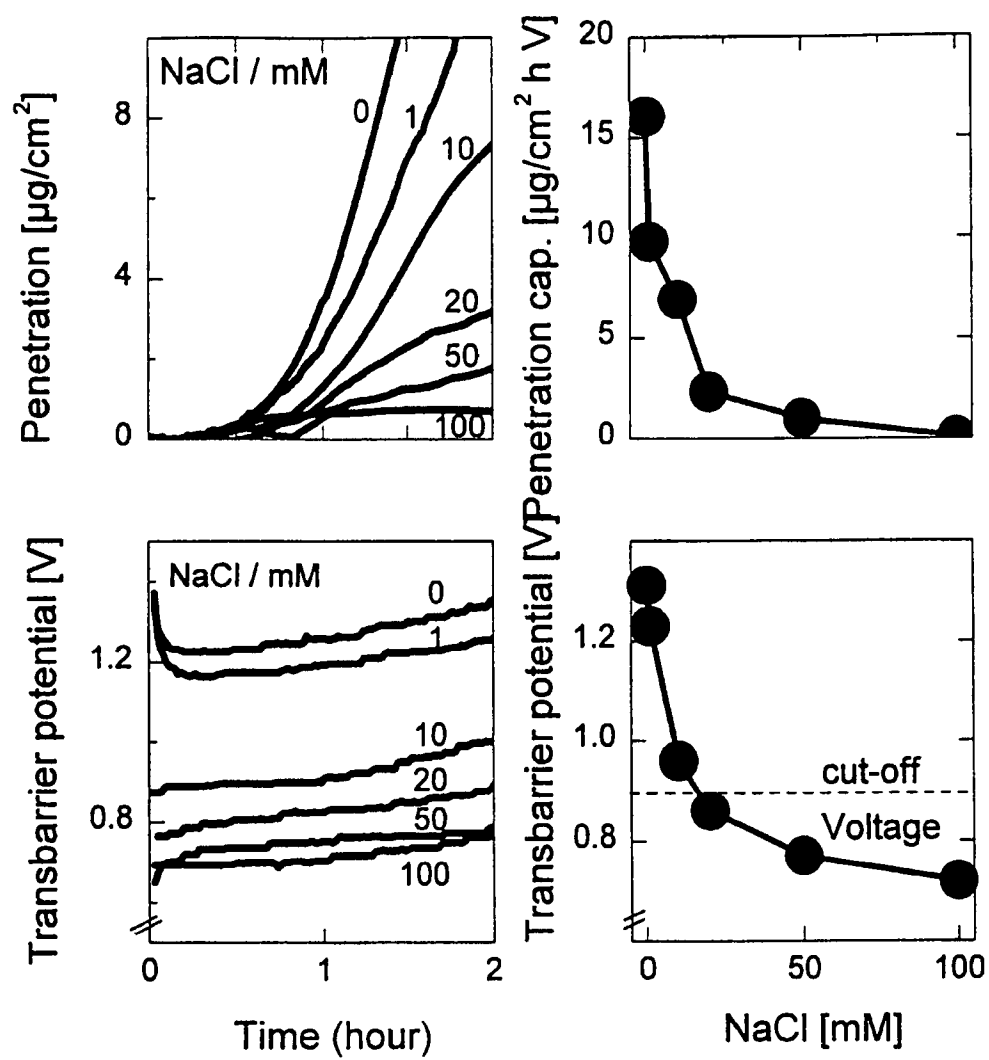


Figure 8

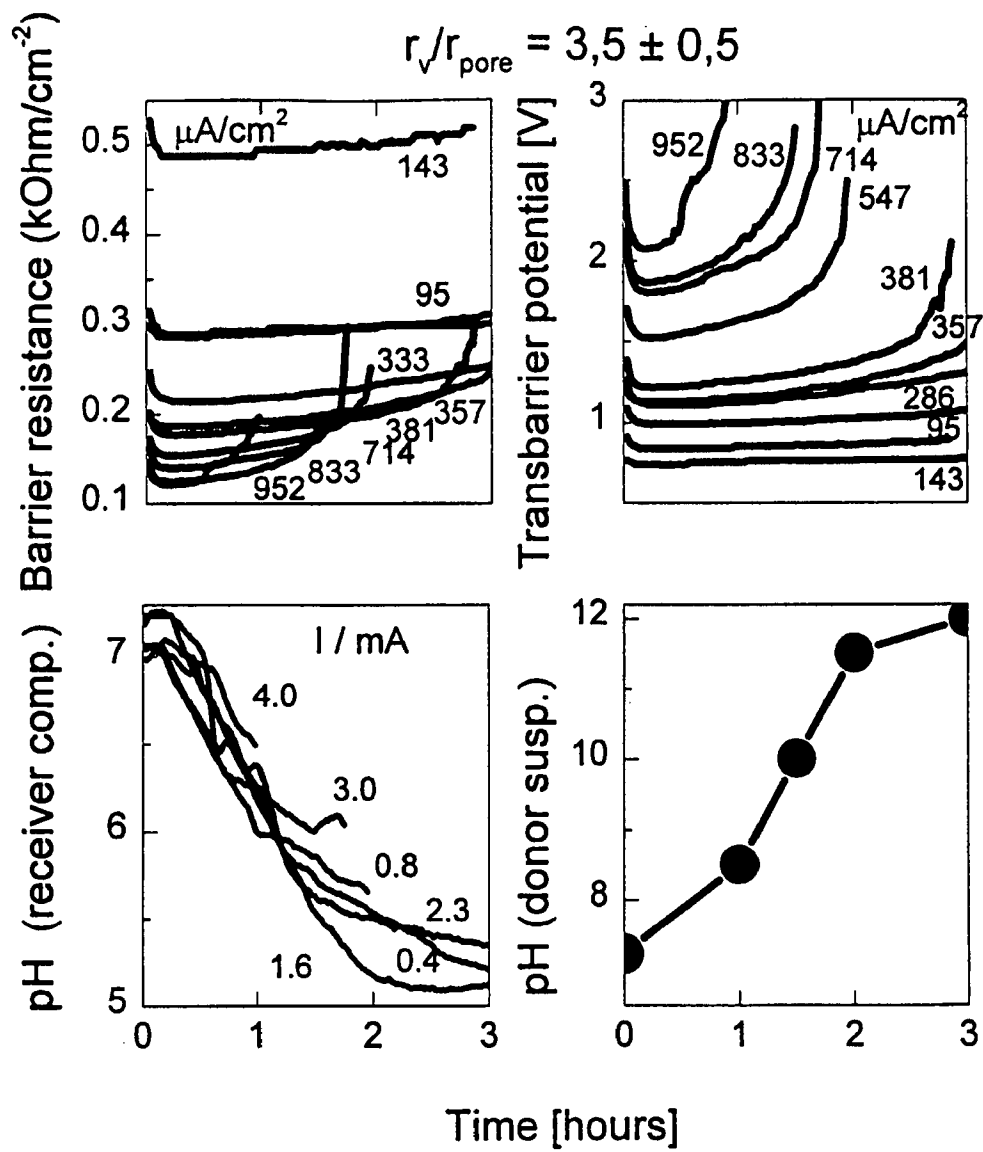


Figure 9

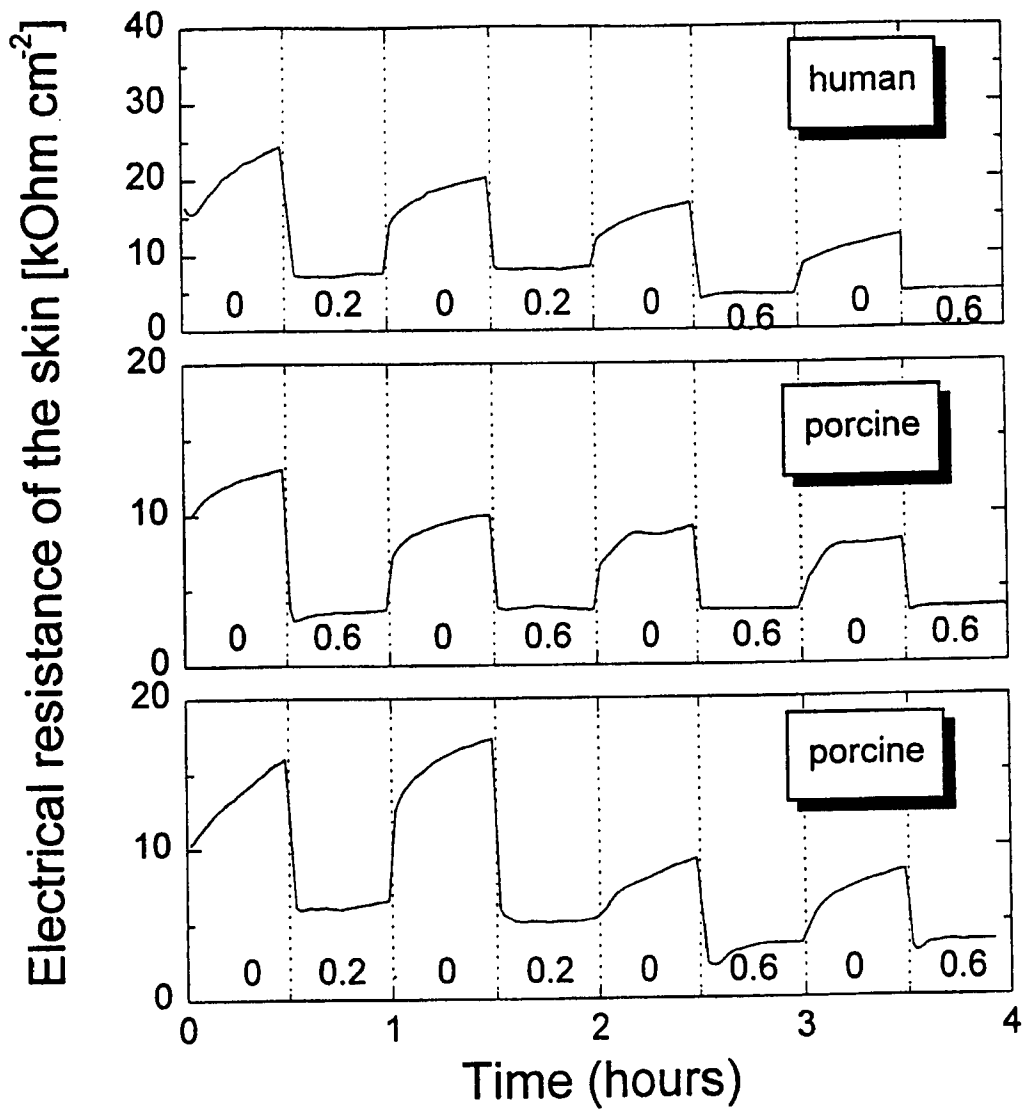


Figure 10

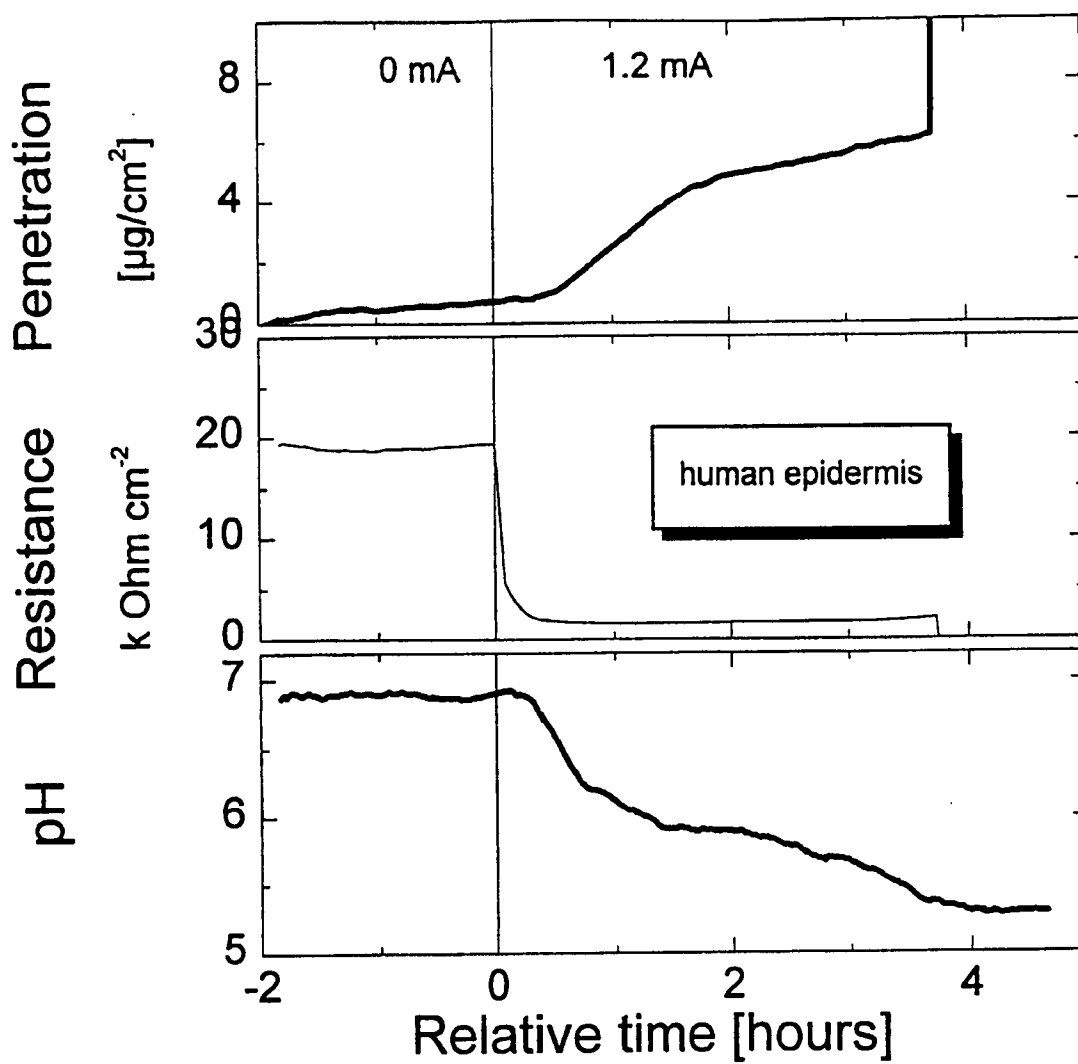


Figure 11A

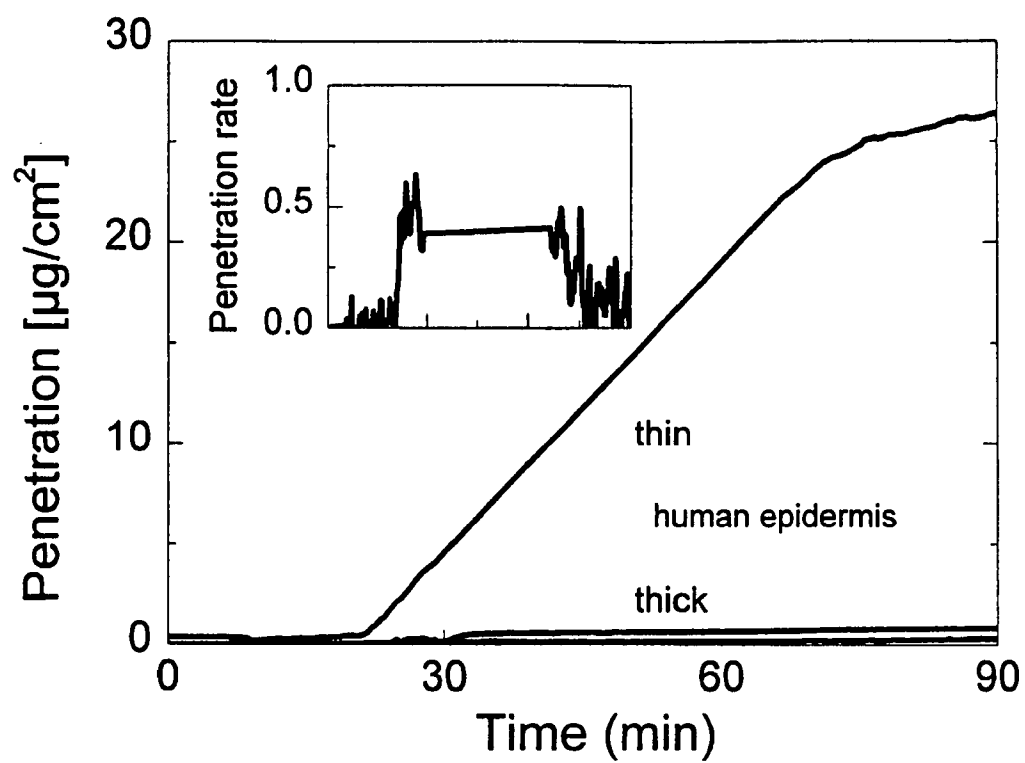


Figure 11B

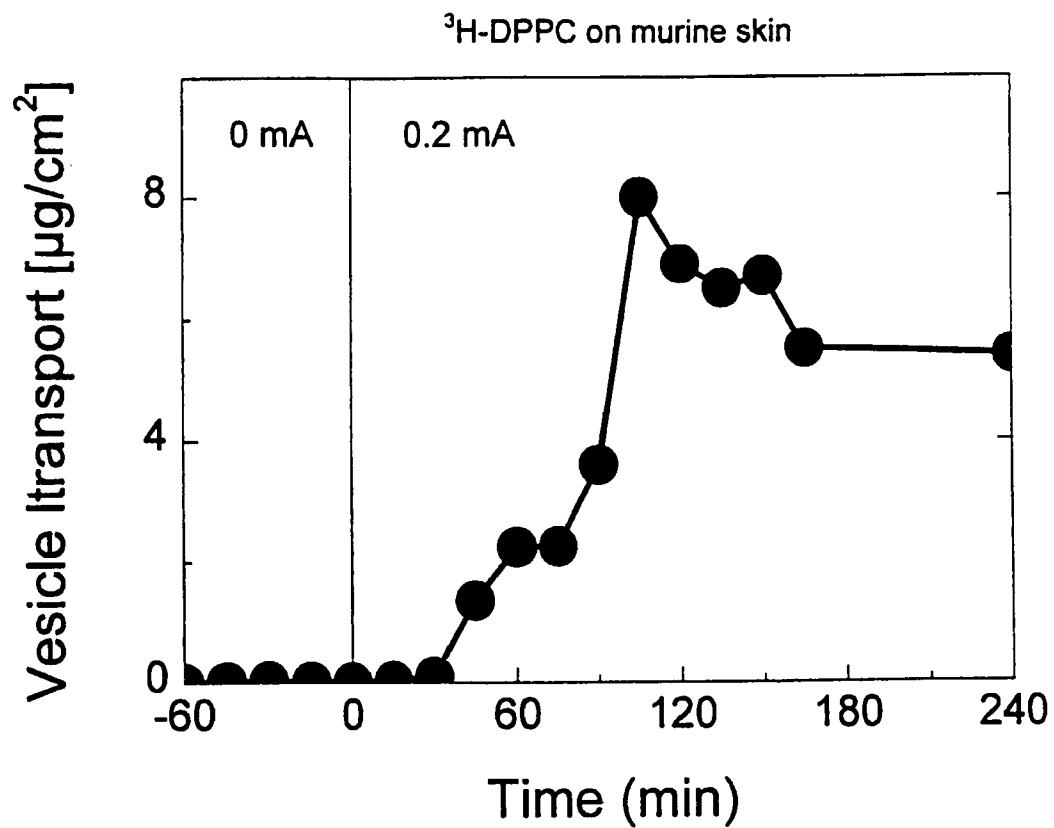


Figure 12

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 98/05539

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/00 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 552 666 A (KAO CORPORATION) 5 April 1985 see page 17; example 4 see claim 1 ---	1-22
A	EP 0 475 160 A (CEVC) 18 March 1992 cited in the application see page 37 - page 40; examples 32-49, 62-98 --- -/--	1-22

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"Z" document member of the same patent family

Date of the actual completion of the international search

28 April 1999

Date of mailing of the international search report

17/05/1999

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Benz, K

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/05539

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	N.B. VUTLA ET AL.: "transdermal iontophoretic delivery of enkephalin formulated in liposomes" JOURNAL OF PHARMACEUTICAL SCIENCES , vol. 85, no. 1, January 1996, pages 5-8, XP000543850 Washington (US) cited in the application see the whole document -----	23-39
A	CHEMICAL ABSTRACTS, vol. 126, no. 8, 24 February 1997 Columbus, Ohio, US; abstract no. 108828k, S.B. KULKARNI ET AL.: "transdermal iontophoretic delivery of colchicine encapsulated in liposomes" page 1034; column 2; XP002101479 see abstract & DRUG DELIVERY, vol. 3, no. 4, 1996, pages 245-250, -----	23-39
A	WO 94 17792 A (AFFYMAX TECHNOLOGIES N.V.) 18 August 1994 see page 26; example 5 see claims 1,7-10,15-17 -----	23-39

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No

PCT/EP 98/05539

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